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THE GROWING AND PROCESSING OF WHOLE COTTON

By Frank K. Cameron

WHOLE COTTON

Definition. The cotton plant, as with any other organism, can be forced to early maturity by crowding it during the adolescent or growing period. One does not obtain as fine an individual plant as when one follows the usual practice of wide spacing. Although the number of bolls on the individual plant may be smaller with crowding the number of plants per unit of area will be greater, and the total production of lint and oil will probably average as much as with planting in the usual way. With close planting, the plants mature within a period of 25 to 30 weeks after planting. There will be both mature and immature bolls, but the maximum weight of cellulose and of oil will have been reached, while the percentage of moisture will have closely approached the minimum. If the plants be cut now, they may be baled, and further drying proceeds without deterioration of the product.

Cotton so harvested is known as whole cotton. It consists of lint, seed, cusps, and stems. Most of the leaves are lost in the harvesting and baling. They constitute but two per cent. or less of the dried plants. They contain coloring matters, difficult to eliminate and the loss is advantageous. Seed cotton (lint and seed) comprises 60 per cent. of the mass of whole cotton, the remainder about equally divided between cusps and stems.

The Purpose in Growing Whole Cotton. Ordinarily, cotton is grown for the seed cotton, the rest of the plant being discarded as of no commercial value. From the seed cotton are recovered lint, linters, hulls, oil, and a meal, for each of which a market has been developed. The lint is nearly pure cellulose, but the cost of producing it is too high to permit it being used by the cellulose industries. Linters, or the short fibers cut from the seed after the lint has been removed by ginning, are likewise a quite pure cellulose, but the limited production and relatively high cost restricts its use to a few special products. The hulls removed from the seeds, before extraction of the oil, and the meal or press cake left after extraction are by-products, but the oil itself has an importance comparable to that of the lint (15).

Industrially, the growing of whole cotton is a very different enterprise, in purpose and method. It envisages the production of a *cheap* source of cellulose,

at the same time conserving the oil from the seeds. To achieve this purpose, planting, cultivating, and harvesting procedures are modified and in some details are actually different. The oil is extracted with a solvent instead of by a cold press. The processing of the residue for alpha-cellulose is quite different from the ginning and keir boiling of lint, and the end product differs markedly in physical characteristics, although essentially the same, chemically.

THE GROWING OF WHOLE COTTON

The Seed Bed. A well prepared seed bed is of the first importance. After a first preparation, it is desirable to give any weed, and grass seeds and roots time to germinate and start growth. A light but thorough discing or harrowing will then uproot them and they will be killed pretty completely if the weather be dry. The cotton can then be planted with assurance of establishing a stand without undue interference from foreign growths.

Fertilizers. On many soils an application of commercial fertilizers will be necessary to secure a satisfactory crop. Apparently, a generous application of a potassium salt is desirable. What mixtures and amounts would prove most desirable may be judged from the practice of the region. The common practice of a top dressing of sodium nitrate after the bolls commence setting is not justified, for a prolonged plant growth is not desired. If animal manures be used they should be disced or harrowed in when the seed bed is first prepared to give opportunity for the eradication of the weeds coming from the seeds in the manure.

Varieties. Seven or eight varieties have been grown for whole cotton, but not enough experience has been gained to justify a statement that any one of these is preferable. The variation in yield has been as great between separate plantings of any one variety as between the varieties themselves. Early maturing and prolific boll-bearing are the qualities most desirable, while size of individual plant and length of staple are of minor importance. A given variety will produce quite a different individual plant grown as whole cotton from what it would have produced if grown in the usual way. It will require time and the services of skilled plant physiologists to solve the problem of the "best" (12). Apparently, any variety known to be adapted to a locality can be used with good results.

Seed. Delinted seeds are to be preferred and with some mechanical planters they may be necessary. Our own experience is confined to seeds delinted by stirring them in concentrated sulfuric acid, a messy but not difficult job. Washed free of acid and air dried, they proved satisfactory in all respects. The Texas State Experiment Station has given much attention to delinting and delinted seeds are on the market.

Planting. Late planting is desirable some weeks later than is usual with cotton for the particular locality. The primary consideration is to have all weeds and objectionable growths so far developed that they can be completely eliminated with the preparation of the seed bed. If a following crop is to be planted when the cotton has been harvested, this must be considered in determining the date. That it is desirable to have a following crop admits of no

dispute, preferably one not requiring clean cultivation. A spacing of 4–6 inches between plants would probably be ideal but appears impractical with mechanical planting devices and 4 to 5 plants per foot have been the usual experience so far. Eight inches between rows has proved to be necessary to permit the passage of the cultivation tool and a greater width if tractor drawn cultivators are to be used. We have found 12 inches practicable. Broadcasting has been tried by Hall, at the Pee Dee Experiment Station near Florence, S. C., with some success, but experiments at the Dockery Farms in Richmond County, N. C., were failures, grass making a more rapid growth than the cotton. Attempted removal of the grass by harrowing tore up so much of the cotton that the experiments were futile.

Cultivation of whole cotton differs from that employed with cotton grown in the usual way in that no "chopping" or thinning of the plants in the row is called for. When the first cultivation should be made, is determined by the condition of the surface, the height of growth of the cotton plants and particularly by any invasion of grass and weeds. Commonly, a second cultivation seems called for, and in some cases a third. But this last may be unnecessary since the ground soon becomes shaded by the cotton plants, keeping the soil surface in good physical condition and inhibiting the growth of grass and weeds.

Soil Character. Much depends on the soil characteristics as well as the plant growth. In a recent experiment a planting on a rough stony soil, ill suited to cotton and difficult to cultivate, the cotton did not develop sufficiently over much of it to shade it and grass invasion was relatively bad. A mile away on a good average soil planted at the same time, the ground was completely shaded in a few weeks and a second cultivation amply met requirements.

HARVESTING OF WHOLE COTTON

The development of a plot of whole cotton has been followed, in a number of instances, by weekly or periodical laboratory analyses. It was found that the data obtained after 10 or 12 weeks' growth are significant. From then on the oil and cellulose content increased until about the 25th or 26th week they have attained their maxima, little or no percentage increase resulting from further growth. Ash and water content, on the contrary, have dropped to minima values which persist for several weeks longer. The individual plants carry 12 bolls, on the average, which are ripe or approach ripeness, with some further immature ones. Leaves are wilting and stems are hardening. Samples have been gathered by selecting an average plant from the plot at regularly placed intervals, quartering and making moisture determination as quickly as possible, while a dry sample was analyzed by standard methods.

Control by laboratory analyses would not be available to the practical grower ordinarily, but the appearance of the plants is sufficiently characteristic to determine when it would be time to harvest. Twenty-six weeks of growth is not regarded as determined arbitrarily. The "best" growing period may be modified by climate, season, soil, variety, cultural treatment or other factors and the judgment of the grower is no less important than in any other agricultural operation.

The first experiments on growing whole cotton were made at the Dockery

Farms near Rockingham, Richmond County, N. C. On this plantation was a gin, with baling equipment and storage space either under cover or on open platform. The cotton was cut with sickle or scythe, piled in small shocks for curing and then hauled to the gin. The next crop of cotton was cut with a hay mower, drawn by horse or by tractor and baled almost immediately in the field by a portable baler. The bales prepared at the gin house were of the dimensions of a standard bale of ginned cotton, but weighed well over 600 pounds a bale, while the bales put up in the field could be made to vary from 125 to 175 pounds, tied with wire as with hay, avoiding the use of straps and bagging. They were more convenient to handle and less expensive.

The cotton cut by the ordinary mowing machine gathered considerable amounts of earth and dust which persisted in adhering through the baling and subsequent operations. It was found later that this silicious material could be quite completely removed in processing but it would be better to avoid it. The John Deere Plow Company carried out an experiment with a tractor drawn assembly. A cutting bar immediately preceded an escalator upon which the cut plants fell with little or no contact with the ground. From the top of the escalator the plants fed into a hay baler, and the bales fell on a trailer. A very clean product was thus sent to storage. There was little or no "shattering" of lint from the bolls, unavoidable with the usual type of mower. The operators estimated that with this equipment the cotton can be harvested for 19 cents per acre. The International Harvester Company has independently given the same figure for harvesting with an assembly of their designing. The Hogan brothers have estimated that the cost of harvesting and storing should not exceed 20 cents an acre.

These assemblies can be readily disassembled and the component parts utilized for other purposes about the farm. The acreage covered in a given time would be the same as for hay and depend on the nature of the terrain and very much on the size and the shape of the field. Short rows requiring much turning of the equipment is the only serious obstacle to efficiency.

STORAGE OF WHOLE COTTON

Bales of whole cotton have been stored for 6 years both under cover and in the open and then found unharmed when broken open. But this same cotton after being extracted with organic solvents will "sour" or mold if left in water-moist condition, as with any plant residue. Twice, insects have invaded the stored cotton, but their depredations were confined to the pith in the stalks and had practically no significance for oil or cellulose content.

It appears settled that long time storage has no deleterious effect on the cellulose. So much is not claimed for the oil. No definite evidence has been found of loss or deterioration due to long storage, but the observations have been few. The necessity of removing coloring matter before or after extraction of the oil has prevented detection of hydrolysis or disintegration.

THE COST OF GROWING AND HARVESTING WHOLE COTTON

Mr. Nicholas W. Dockery (6) of Rockingham, N. C., who is responsible for much of the earlier laboratory work on whole cotton has had also more experi-

ence in growing it than any other individual. He has reported a cost of $15 to $16 per acre for growing and harvesting, omitting only an item for rent of the land. A "breakdown" of the figures into separate items involves, however, estimates, since the operations were carried on in connection with the large cotton plantings at the Dockery Farms. Milne (14) attempted detailed estimates from these and similar data concluding that they are probably truly representative of actual practice. In the spring of 1940, the International Harvester Company made a grant to cover the cost of operations in growing whole cotton, using only machinery methods. The co-operation of Mr. Don Matthewson, the County Agent for Orange County, was secured and the Hogan brothers carried out the operations four miles from Chapel Hill. Two plots were selected about a mile apart. One (North Plot) was a good average soil of the vicinity of the Cecil clay loam type. The second (South Plot) was selected largely as a contrast; it was very stony and proved to be ill adapted to machine operations. It was a Norfolk sand in type. Neither, so far as known, had ever been planted to cotton, but only to corn or grass. Half of each plot was planted in rows eight inches apart, the remainder in rows twelve inches apart. On May 19, the plots were harrowed and on May 22 they were planted, using a tractor drawn *Vegetable Planter*. No difficulty was encountered in planting the North Plot, but the stony, rough surface of the South Plot made it almost impossible to keep the tractor drawn seeder in line on a relatively large part of the area. The plants on both plots made a good start. A few and only light showers wet the plots between planting and harvesting. Corn, in the field adjacent to the North Plot, gave a very poor yield and that in the field surrounding the South Plot was a failure. Nevertheless, the cotton plantings made good progress and on the North Plot, so shaded the ground within three weeks that only an occasional foreign plant appeared. On the South Plot growth of the cotton plants was relatively slow after the first fortnight, never completely shaded the ground and there was a considerable invasion of crab grass; finally, nearly half of the plot was abandoned as not justifying the expense of cultivation. Apparently the bolls set sooner on the plants in the South Plot than in the North Plot, but a comparative count was hardly sufficient to justify a definite statement. Cultivation of both plots was made on June 10 with a tractor drawn *Vegetable Cultivator* and later with a *Rotary Hoe*. Probably two cultivations only, properly spaced as to time, would have sufficed for the North Plot. Because of an impending storm, the harvesting was several days earlier than planned and without waiting for the assembly to which reference has been made above. Hastily, the plants were mowed with horse drawn scythe, gathered with rakes and forks, baled in the field and hauled to a barn. In Table 1 are assembled cost data computed from the records of the Messrs. Hogan. Possibly all, and certainly some, of the items are higher than they would be on a larger scale operation better adapted to mechanized equipment. The items for mowing and baling are very far from the 20 cents an acre estimated as practicable.

A composite sample of the whole cotton from the three plots was analyzed and gave: total cellulose, 53.5%; oil, 8.4%; ash, 5.2% (mostly adhering soil); and water, 32.9%. Water and ash are unduly high.

It would seem that the Dockery estimate of $15 to $16 per acre has been justified. There remains to be added a charge for use of land, machinery, taxes, superintendence, etc., or say $20 per acre *in toto*. But our concern is with the cost of a unit weight of whole cotton; and the yield per acre varied in ways that often appear inexplicable. We are quite unable to explain that in the experiment on the North Plot the acre planted to 12 inch rows gave nearly a ton and a half yield above the acre planted to 8 inch rows. However, with the experience so far attained, it seems safe to say that a yield of 5000 pounds per acre should be obtained readily and has been exceeded more often than not.

TABLE 1

Yields and Costs for Whole Cotton

	NORTH PLOT		SOUTH PLOT
	8″ rows	12″ rows	12″ rows
Preparing ground	$0.60	$0.60	$0.60
Seed	2.75	2.75	2.75
Fertilizer	11.08	11.08	11.08
Planting	0.15	0.15	0.15
Cultivating	1.95	1.95	1.95
Mowing, baling and storing	3.40	6.00	1.60
Total operating costs	19.93	22.53	18.23
Rent of land	3.00	3.00	3.00
Total production costs	22.93	25.53	21.23
Whole cotton, pounds	4229	6978	3200
Whole cotton, cost per ton	10.84	7.32	13.27
Cellulose, pounds	2263	3733	1712
Oil, pounds	355	586	269

THE COMPOSITION OF WHOLE COTTON

Whole cotton may be regarded, conveniently, as a mixture of 20 per cent. stems, 20 per cent. cusps, and 60 per cent. seed cotton. Stems and cusps are wood, containing cellulose and the usual accompanying substances of ligneous growth. Seed cotton comprises lint, an almost pure alpha-cellulose, and seed meat surrounded by the hulls.

A mixture of waxes coats the lint and perhaps other parts as indicated by the fact that the de-leafed plant does not ferment before removal of the waxes. The hulls are said to contain 40 per cent. alpha-cellulose (1). The cellulose content of the seed meat alone has not been determined, but it has been for the whole seed. The seed meat is the source of cotton seed oil, a mixture of glycerol esters of unsaturated long chain paraffin acids. It contains also, to some extent, waxes. The processing methods are such that gossipol and other deleterious content of the seeds are excluded from the oil quite completely. The content of oil is about 8.5 per cent. of the air dried whole cotton.

The wood portions, stems and cusps, contain lignin which does not at present have any commercial significance and a small percentage of an aggregate including some highly colored substances. These last, unless they be first removed,

tend to accumulate in the solvent extract and make the refining of the oil a difficult task.

In addition to the oil, the commercially desirable component of whole cotton is cellulose; or, more properly, alpha-cellulose. It can be obtained from whole cotton by any of the procedures used in treating commercial woods, but there are definite advantages in the dilute nitric acid method (7).

The percentage of cellulose in whole cotton has been found to vary from about 45 to upwards of 55. This variation is to be expected since the proportions of stems to fruit is dependent upon variety, soil, seasonal and cultural vicissitudes, insect depredations, and the many factors affecting growth. In an exceptional case, when the whole cotton per acre reached the very large figure of 17 tons per acre, the production of cellulose per acre was about average although the percentage of cellulose in the plant was very low; the major portion of the crop was stems with comparatively few bolls. In general, with a good average crop, the content of cellulose will be above 50 per cent. It is not to be forgotten that, from the producer's viewpoint, it is the yield per acre that is significant, for it costs as much or more to bring a poor crop to harvest as it does a good crop.

The "total" cellulose as obtained by any of the procedures as yet proposed or used is not a pure product. Possibly in the plant itself, but certainly in the processing of it, there is some degradation and breaking of the large molecular aggregates. Hence, arbitrary methods have been devised for testing the cellulose. The sample of pulp or total cellulose is agitated in an aqueous solution containing 17.5 per cent. sodium hydroxide. That which does not go into solution or colloidal suspension is designated alpha-cellulose and it alone is of any present commercial value. From the mother liquor a precipitate is formed on the addition of a slight excess of acetic acid. This precipitate is designated as beta-cellulose, is not of itself commercially valuable, but its determination may be useful in judging the suitability of the pulp for certain purposes. The difference between the total pulp and the sum of the alpha- and beta-cellulose is called gamma-cellulose. Since the proportions of alpha-, beta-, and gamma-celluloses are generally held to be more or less characteristic of the origin, pulps have been prepared from delinted seed, cusps and stems as well as the whole cotton and the percentage of alpha-cellulose determined in each (6).

The results for the several pulps were: seeds, 83.3; cusps, 75.8; stems, 82.9: and for whole cotton, 97.3 (4). In an earlier study on less favorable material and different pulping procedure, the percentage of alpha-cellulose in five pulps was found to vary from 86.7% to 94% (10). From these figures there can be estimated the proportions of cellulose in whole cotton derived from the several components. The data are assembled in Table 2. From these data it would appear that the whole cotton would yield 45.6% total cellulose of which 89.5% is alpha-cellulose. Probably the percentage of lint has been estimated too conservatively, for an analysis of the whole cotton, from which these components were obtained, gave 53 per cent. total cellulose of which 93.3 per cent. was alpha-cellulose. The tabulated data indicate that 53 per cent. of the total and 59 per cent. of the alpha-cellulose in whole cotton comes from the lint.

Cellulose pulps prepared from wood contain other hydrocarbons, such as pentosans. For some uses, the presence of these may be desirable, but they are more often regarded as impurities and a standard method has been designed for estimating them. Inorganic constituents, or ash, should be and usually are low. With commercial pulps, the ash sometimes contains lime absorbed from the water supply. With whole cotton, the ash is mainly silica from adhering soil particles, which can be removed readily, when desired, in the process of pulping.

In addition to the composition of the pulp, the "copper number" and the viscosity of a suspension in cuprammonium solutions are determined by standard procedures, the data being regarded as indicative of the extent of degradation

TABLE 2

Distribution of Cellulose in Whole Cotton and in the Several Components

COMPONENT	% OF COMPONENT	% TOTAL CELLULOSE FROM COMPONENT	% OF ALPHA-CELLULOSE FROM COMPONENT
Lint	24	24.0	24.0
Seed	36	5.6	4.1
Cusps	20	8.0	6.1
Stems	20	8.0	6.7

TABLE 3

Characteristics of Cellulose Pulps from Components of the Cotton Plant

SOURCE OF PULP	STEMS	CUSPS	SEEDS	WHOLE COTTON
Alpha-cellulose	82.48%	82.34%	83.05%	84.21%
Beta-cellulose	1.10%	2.89%	3.21%	10.61%
Gamma-cellulose	16.42%	14.77%	13.74%	5.18%
Pentosans	8.78%	9.30%	14.81%	4.44%
Copper number	1.89%	2.07%	2.03%	1.15%
Cupra Ammonium Viscosity	14.4*	17.3*	21.1*	7.3*
Ash	0.93%	0.23%	0.18%	0.26%

* Centipoise.

incidental to processing. In Table 3 are assembled the results of the determination of these characteristics for a sample of whole cotton and its components (7).

These data indicate that a pulp from whole cotton is comparable to commercial pulps from woods prepared by standard practices; and awaiting the pragmatic test of actual use, it must be considered as equally important.

PREPARATION OF WHOLE COTTON FOR PROCESSING

Grinding. The seed must be broken to permit effective contact of the oil with organic solvents. The stems and cusps must be reduced to small fragments for effective action of pulping solutions. Dockery found that the common form

of hammer mill used on farms for the preparation of cattle feed will pulverize satisfactorily dry whole cotton. A mill better adapted to the purpose was obtained later from the O. B. Wise Manufacturing Company of Knoxville, Tennessee. This mill is fitted with two screens; one with 0.26 inch openings, the other with 0.06 inch openings. The finer screen has seldom been found necessary nor particularly desirable.

The mill product is a fluffy mass, with harder particles imbedded or entangled in lint. *Apparently*, the lint predominates, although actually but a quarter in weight of the whole mass. A more or less efficient separation of the lint is possible by air elutriation during or subsequent to the grinding but that is not now considered desirable.

Removal of Coloring Matter. Dark colored substances may be extracted from the woody components of whole cotton by organic solvents or aqueous solutions of alkalies. Because of its high dispersing power, sodium sulfide is very effective. Warming the extracting solution increases its effectiveness and several repeated seepings will remove more or less completely the coloring matter. It has been found that a single seeping over night, in a two per cent. solution of sodium sulfide in sufficient volume to cover well the solid mass, gives a satisfactory result. The wet mass was thrown on a wire screen, the mother liquor pressed out and the residue washed twice with clean water. With each washing the water is removed as far as possible by pressing the mass on the screen. The effluent has the appearance of the "green liquor" in a Kraft pulp mill. The pressed residue will be sufficiently dry to be extracted with a low boiling paraffin oil (18).

THE RECOVERY OF COTTON SEED OIL

The physical characteristics of pulverized whole cotton precludes the extraction of the oil by pressing, which is the more common American practice when seeds alone are the source of the oil. Solvent extraction must be employed, a device which is now receiving much attention in the United States and which has long been common practice in Europe (18). All the common fat solvents have been found effective in extracting the oil. Because it is not flammable and is cheap, carbon tetrachloride has received much attention. But it decomposes on heating, corrodes metals, and "tenders" the cotton fibers, i.e. causing them to become brittle and easily pulverized. The most satisfactory solvent has proved to be a low boiling (70°C.–75°C.) petroleum oil. This solvent dissolves wax coating the lint, which is not itself objectionable; it would extract much coloring material from the stems and cusps, unless it be first removed as recommended above; it does not extract gossipol (16).

On recovery of the solvent by evaporation, a rather dark colored residue of oil remains. Some samples, on standing several days have deposited a small quantity of fluffy solid, which has not been identified. The oil, shaken with carbon black and filtered, yields a water white filtrate. A sample which has been exposed to the light for upwards of three years is yet entirely without color. In Table 4 are assembled, for comparison with the standards prescribed by the

American Oil Chemists' Society (2), the results of tests on a crude oil extracted from whole cotton which had not been pretreated with a solution of sodium sulfide, a crude oil from whole cotton which had been pretreated, and the refined oil prepared by shaking the second crude with carbon black and filtering (18).

Satisfactory oils have been obtained by decolorizing or "bleaching" the hydrocarbon extract, before volatilizing the solvent. There are advantages, apparently, in doing this; the yield of oil is larger.

TABLE 4

Comparison of Characteristics of Oils from Whole Cotton with Standard Specifications

	STANDARD SPECIFICATIONS A.O.C.S.	CONSTANTS DETERMINED FOR		
		Crude Oil from Whole Cotton	Oil from Na_2S Pretreated Plant	Refined Oil from Na_2S Pretreated Plant
Density at 25°C	0.916–0.918	0.885–0.890	0.893–0.900	0.908–0.912
n_D at 25°C	1.468–1.472	1.475–1.479	1.474–1.476	1.473–1.475
Saponification Number	190–198	178	188	198–201
Iodine Number	106–113	88		101–105

COST OF RECOVERY OF OIL

Crude cotton seed oil is about 15.5 per cent. of the seed, and the average recovery of refined oil from crude is 92.5 per cent. in American practice (15). The prewar annual output in the United States was about 1,450,000,000 pounds, surpassed as an edible fat only by butter and lard. The residue from the refining is used for soap stock. The recovery of crude oil by solvent extraction in Europe yields a higher percentage; but the oil is more highly colored, and there would probably be some loss in weight and increased cost to bleach it to American standards. The crude oil obtained from whole cotton resembles closely that obtained in Europe from cotton seed.

It is conceded generally that the cost of equipment and installation of a mill for solvent extraction including solvent recovery would be no greater than for a press mill of the same capacity and that operating costs would be about the same. A mill to handle whole cotton may not be strictly comparable, because of the larger mass of material to be extracted.

For the decade preceding 1937 the average sale price for crude oil was 6.54 cents per pound, and was 7.86 cents for refined oil. For the past two years the figures have been above 10 cents for crude and 12 cents for refined, which reflects but is not accounted for by an increase in the price of seed.

Unfortunately, the available cost sheets of neither United States nor European mills disclose the cost of extracting the oil. Probably, it is less than 3 cents per pound. To this should be added the cost of raw material, and the usual fixed charges for interest, taxes, repairs and renewals, etc. But if present prices are to prevail, the spread between sales prices and production costs is more than sufficient to pay for the growing and harvesting of the cotton and the recovery

of the oil. The *practical* significance is that the degreased residue going to the pulping mill may be priced as low as the handling charge.

It is assumed, generally, that whole cotton would be grown primarily as a raw material for a pulp mill, and that oil would be recovered as a by-product. The question is posed, how far should a pulp mill be concerned in an oil mill? If the preliminary treatment to remove coloring matter be adopted, it would be advantageous to have the solvent extraction closely adjacent to the pulping operations. It might be desirable to ship the crude oil to a refining plant, as do some of the mills recovering oil by pressing. A recovery of oil by a pulping enterprise using whole cotton would be no more anomalous than the recovery of turpentine and resin by a mill using pine or tannins by a mill using spruce and hemlock.

PULPING WHOLE COTTON

Dried, ground whole cotton, which has not been degreased probably can be pulped satisfactorily. It has been done, tentatively, in attempts to recover the oil in the form of soaps from alkaline pulping liquids.

The degreased whole cotton is admirably adapted to pulping. Many pulps have been prepared by the sulfite, sulfate, and soda processes. Attempts to use these methods but without increased pressure, resulted in pulps which sometimes contained fragments of stems which were not thoroughly disintegrated. These fragments would disappear with "beating" of the pulp and could be avoided more or less completely by finer grinding or by seeping in an alkaline solution preparatory to extracting the oil.

In view of Pompilio's success with wheat straw (17), attempts have been made to pulp whole cotton with chlorine, but with unsatisfactory results. The time of treatment, temperature or pressure necessary to pulp the woody fragments of the stems and cusps resulted in marked degradation of the cellulose (13).

Satisfactory pulps have been obtained by treating whole cotton with a five per cent. aqueous solution of nitric acid, at atmospheric pressure. Although this reagent has been employed in the industry to a very limited extent as yet, it commands an increasing amount of attention and laboratory studies seem to be uniformly favorable to its use (7).

THE COST OF PULPING WHOLE COTTON

It has been noted above that after extraction of the oil, the residue of whole cotton could be delivered to the pulp mill at no cost other than a handling charge, and is, therefore, the cheapest possible source of cellulose. It must be expected, however, to carry part of the cost of its preparation. The economic or business data are not available on which to base a reasoned statement, other than that whole cotton can compete with any known source of cellulose.

Aside from the extraction of the oil, the principle item in the preparation of the whole cotton is the grinding, an operation costing no more than the barking and chipping of wood. The wood of the cusps and stems is easily pulped, and the amount of pulping reagents required is no more than for the usual woods.

Less bleach is required. High pressures not being necessary, degradation should be less. In consequence, the cost of producing a high grade cellulose from whole cotton can not exceed and should not equal the cost of production from any competing source.

CELLULOSE DERIVATIVES FROM WHOLE COTTON

Since degreased whole cotton is so readily pulped, attempts have been made to avoid or by-pass pulping, and make cellulose derivatives directly. Nitrates have been made readily, acetates with difficulty, but no success was achieved in efforts to make cellulose ethers (9). Admitting the possibility of more favorable results with larger sized experiments, the results indicate the economic desirability of first preparing the cellulose by a recognized pulping procedure.

USES OF CELLULOSE FROM WHOLE COTTON

The cellulose from whole cotton and from lint, stems, and cusps separately has been studied by means of X-ray techniques and found to differ in no way from the cellulose obtained from the commercially used woods (21). Dark field studies of the fibers with the microscope failed to show significant differences (5). Esters, ethers, xanthates, and dispersals in cuprammonium solutions, and alkali solutions, have been studied in comparison with like preparations from commercial celluloses, without essential differences being observed. It may be granted that the scale of the experiments is too small to carry conviction to the industrial expert. But they are an ample justification for the larger sized tests he may properly desire. It has been objected, that for certain uses, the variation in length of fiber in the cellulose from whole cotton would bar its use. This may be true, for certainly the cellulose from seed meats differs in fiber size from the cellulose from lint. It, however, comprises but a small fraction of the whole, and the mixture of fiber from lint, stems, and cusps seems to be no more diverse in character than the mixtures of linters and wood cellulose commonly used in the industry.

SUMMARY

The salient facts obtained in a series of studies in field and laboratory show the possibility of utilizing the cotton plant as a raw material for the production of oil and alpha-cellulose. We can have *available* a valuable, satisfactory and adequate raw material to supplement on the one hand our supply of fats and on the other hand to supplement existing commercial sources of cellulose. Before these conclusions can be accepted, it is evident that larger sized experiments must be made and preferably commercial or semicommercial trials by the pulping industry.

The "unit" processes involved in the suggested processing of whole cotton for the recovery of oil and cellulose are all well established and no engineering difficulties are involved.

The suggestions advanced in this paper, do not imply that the growing of cotton for lint should be abandoned. There is little likelihood of this in any

foreseeable future, although there is general agreement that it should be restricted in the interest of the national economy. There are areas where cotton is now grown, but with scant hope of it ever being done profitably, where whole cotton might well offer economic advantages and could be substituted without too radical changes in the farmer's accustomed practices.

Finally, the growing of whole cotton for oil and cellulose, and the growing of cotton for lint and the usual by-products should be regarded as distinctly different industrial enterprises. The one point of contact is that both produce oil; and for this there is no prospect of a glut.

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MITOSIS AND CELL DIVISION IN PONTIGULASIA VAS (LEIDY) SCHOUTEDEN

By A. B. Stump

Plate 1

In a previous paper, the reproduction of *Pontigulasia vas* was briefly considered in relation to the materials needed for the test. The present work was undertaken to give a more complete description of binary fission in this representative of the *Difflugiidae*.[1]

MATERIALS AND METHODS

The specimens of *Pontigulasia* used in this investigation were collected from a number of ponds in the vicinity of Charlottesville, Virginia, and the Mountain Lake Biological Station, Mountain Lake, Virginia. After identification the animals were isolated in Syracuse watch glasses with clean *Spirogyra*. These cultures were kept in moist chambers. By frequent changes of water and the occasional addition of fresh alga they were easily kept in good condition over the four or five days until used. The pH value ranged from 6.3 to 6.9 in these cultures. The temperature range was wide but this did not seem to be a critical factor as long as the changes were slow and the temperature did not rise above 20°C. A special effort was made to keep all such cultures free from sand or similar materials which could be used by the animals to form their shells.

When *Pontigulasia* are cultured without sand or other shell materials they are unable to divide (Stump, 1936): they will, however, form the peculiar pseudopods used to collect such materials. Animals with these pseudopods were found under the wide field binocular microscope and removed to separate dishes for close observation under the 16 or 8 mm. objective of the compound microscope. Finely powdered sand in water was then added and in a short time the animals would begin to collect it. At different stages in the collection and division these animals were fixed. It was found that animals about to divide, as well as those dividing, were very sensitive to movement or bright light and unless care was taken they would discard the sand and halt the division process. Although the shell of these animals and the mass of materials they collect obscure the processes of division to a large extent, it was possible to fix them at different mitotic stages with some accuracy. This was done by fixing the animals at various intervals from the time the collection of sand ceased.

Fixation was carried on with Bouin's, Schaudinn's, Carnoy-Lebrun and Allen's

[1] The greater part of this investigation was made possible by a fellowship at the University of Virginia and for this the author wishes to make grateful acknowledgement. The remaining work was carried on at the Mountain Lake Biological Station of the University of Virginia, Mountain Lake, Virginia, and at Flora Macdonald College, Red Springs, North Carolina. Thanks are also due to Professor B. D. Reynolds of the University of Virginia for his suggestions and assistance.

B 15 modification of Bouin's fluid. Bouin's and Allen's modification gave best results.

The sand grains of the shell of *Pontigulasia* make any examination of the internal structure almost impossible. Sectioning was tried as a solution to this problem but without success except in a few cases where the animals had been forced to use mica for their shells. Even here so many of the sections were lost that the process was given up. The method used in this work was to remove the silica ($Si\ O_2$) of the shell, chiefly sand, by treating the fixed animals with 2% hydrofluoric acid (H_2F_2) in 70% alcohol (Stump, 1936). Because the nucleus of these organisms lies in the center of a mass of cytoplasm containing many deeply staining bodies and food vacuoles, it was found best to embed the animals in paraffine after the acid treatment and then to section them. Embedding and sectioning were carried out in the usual manner except that most operations were done with the aid of a wide field microscope. The thickness of the sections varied between 7 and 15 μ in different animals.

A number of the usual stains were used, such as Heidenhain's iron hematoxylin, gentian violet, and Delafield's hematoxylin. Delafield's hematoxylin diluted one half to one third with water gave best results. It seems that the acid treatment had some effect on the staining reaction.

The effect of the acid on the fixation and staining was determined in two ways. First, by comparing the sections of the mica-shelled individuals already mentioned with those treated with the acid. Second, a large number of *Amoeba proteus* were fixed and divided into two lots. One lot was placed in 70% alcohol; the other in alcohol and acid. Both lots were then stained and mounted, using the same technique. This procedure was repeated, using various fixatives and stains. It was found that the acid had no appreciable effect on fixation but did change the staining reaction in that the usefulness of some techniques was lowered. This was particularly true in the case of Heidenhain's iron hematoxylin.

DESCRIPTION OF MITOSIS AND CELL DIVISION

The general organization of *Pontigulasia* in the resting condition is very similar to that found in *Difflugia* by Goette (1916), Pateff (1926), and others. The shell or test differs from that of *Difflugia* chiefly by the presence of an internal diaphragm between the neck and the fundus (Fig. 1-A). The mass of cytoplasm lies in the fundus of the shell with the pseudopods extending through the diaphragm and neck to the exterior. This cytoplasmic mass contains numerous spherical bodies ranging from 1 to 6μ in diameter. Some of these are food vacuoles while others are of an undetermined nature. The number of these bodies varies greatly in different animals. There are also contractile vacuoles present, usually lying in the outer part of the endoplasm near the diaphragm. The nucleus of *Pontigulasia vas* is vesicular in type. It is almost spherical in shape with a diameter ranging from 18 to 20μ. Within the distinct nuclear membrane are found 15 to 25 deeply staining endosomes. Most of the endosomes lie near the nuclear membrane but some are scattered within the interior

(Fig. 1-B). Forming a background for the endosomes is a more or less diffuse net of lightly staining material. When closely observed this net is seen to be composed of irregular ribbon-like strands, many curved and branched. There are a few granules scattered on the net, but taken as a whole it is not granular in composition. See Fig. 1-A. Most, if not all, of the chromatin of the resting nucleus is in this structure.

For approximately four hours preceding division the animals collect materials for the new shell. The pseudopods during this period are large and irregular in outline, with a wide hyaline area at the ends. Movement is very active at the beginning, and the shell is often held in a vertical position. As the sand grains are seized by the pseudopods, they are passed back toward the mouth of the shell. Grains of all sizes small enough to be moved are thus indiscriminately added to those collected in a mass just in front of the mouth. As the animal moves about, most of the large particles collected early in the process are lost. Thus most of the grains close to the mouth tend to be small. Later the movements become much slower and the larger pieces will be held. As will be seen, this arrangement will affect the position of the sand grains in the completed shell. It appears unlikely that more than a few of the smallest grains are taken inside the mouth. During the collecting period the nucleus remains unchanged. Following the collecting period the psuedopods are withdrawn, and there is a short time of inactivity marking the onset of nuclear changes.

The Prophases.—The early prophases begin with the end of pseudopod formation. The nucleus is enlarged and the endosomes show a slight swelling (Fig. 2-A and B). The net of lightly staining material has now somewhat contracted so that it is darker and more thread-like, though still irregular and often disconnected. There is an increase in the size and number of granules on the net. In a few examples of very early prophase the net tended to form more in the center of the nucleus, leaving a clear area next to the nuclear membrane. This resembles closely the situation found by Pateff (1926) in *Difflugia pyriformis.* At about the same time as the onset of the prophases, the cytoplasm becomes highly vacuolated (Fig. 3-A). This animal had not yet started formation of the new shell although the nucleus is in middle prophase. Note that the fundus is almost completely filled with cytoplasm and a large mass of it projects from the mouth of the shell. Before treatment with the acid, the part projecting was completely covered with sand grains. As a rule, by early prophase the shell formation is well advanced. The formless mass of cytoplasm outside the shell takes on a bubble shape which extends out from the mouth. Once started the bubble enlarges rapidly and the sand grains arrange themselves over the surface as it forms. As Rhumbler (1898) has suggested, the orientation of these grains is probably due to surface tension on the cytoplasmic membrane. This first swelling forms the fundus of the new shell and takes place so that the outer sand grains, collected last, cover this surface. The grains collected earlier are left as a sort of collar about the mouth of the old shell. After a pause the neck is formed, pushing the fundus outward. As this takes place, the neck is covered by the materials of the collar mentioned above. Thus the sand grains

are arranged on the new shell so that the large ones cover the fundus, while the neck is composed of smaller ones. So rapid is this process of shell formation, 15 to 20 minutes, that by middle prophase the new test is usually complete except for the cement which will hold the grains together. At this point the two shells lie mouth to mouth. Fig. 2-A shows a section of an animal at the end of the shell-forming process except for a small part of the neck of the new test. The cytoplasm in the fundus of the old shell is somewhat contracted over that seen in Fig. 3-A, while the cytoplasm outside the mouth of the shell has enlarged and taken definite shape. The many small vacuoles seen in the earlier stage have given way to a few large ones, particularly in the area where the fundus of the new shell will be located. This is the bubble of cytoplasm mentioned above.

In middle prophase there is little change in the cytoplasm and its role in shell formation; the processes already started in early prophase are continued. As has been noted, the animal shown in Fig. 3-A was not typical, in that development of the test had lagged behind the nuclear changes. Nuclear changes in middle prophase are marked over those in the previous stage. The size of the nucleus has increased and the endosomes are swollen and highly vacuolated. The net now is well developed as definite strands with the chromatin granules collecting on them in clumps. In some sections these strands seem to radiate out from the center of the nucleus as in Fig. 3-B; other nuclei at this stage show the strands of the net extending across or radiating out somewhat from one point near the periphery (Fig. 3-C), depending on the position of the nucleus. In middle prophase the nucleus has reached its maximum enlargement.

By late prophase the formation of the shell for the daughter animal is finished except that the sand grains are only lightly cemented together. The cytoplasm appears to be but little changed from the earlier stages though there are fewer vacuoles. Fig. 4-A shows the cytoplasmic features of an animal in this stage. The nucleus in late prophase has begun to shrink and at the same time the clumping of the chromatin in the beginnings of an equatorial plate can be seen. The endosomes are present in this stage, particularly the ones close to the nuclear membrane, but they are small and seem to be disintegrating. See Fig. 4-B. The strands of the net, so clear in middle prophase, are difficult to observe in some cases.

The total time for the prophases averaged 48 minutes with extremes at 40 and 70 minutes. This is much slower than for *Amoeba dubia* as reported by J. A. Dawson, Walter R. Kessler, and Joseph K. Silberstein (1935), or for *Amoeba proteus* by Chalkley and Daniel (1933). Early and middle prophase occupied most of the time; the later stages being quite rapid.

Metaphase.—The cytoplasm in metaphase continues to resemble that of the prophases in most respects. The fundus of the new shell is filled with it, and there is a long pseudopod-like mass extending back to the ball of cytoplasm in the old test. Sections made at this time, such as seen in Fig. 5-A, show that the cement between the grains of the new shell is well formed. The character of this cement was not investigated but according to most authorities in

similar forms it consists of chitin or pseudochitin (Awerinzew, 1909; Zuelzer, 1904). The metaphase nucleus shows a marked decrease in size and as a result the nuclear membrane is wrinkled. The membrane, however, remains unbroken. The position of the nucleus is in the center of the cytoplasm of the old shell, having moved toward the mouth from the preceding stages. Fig. 5-B shows the nucleus of Fig. 5-A in more detail. There is a well formed equatorial plate with 8 to 12 chromosomes lying on the spindle fibers. These chromosomes, while relatively large, appear to have a number of granules associated with them on the spindle so that they are not clearly defined. Under careful inspection the chromosomes seem to be granular in nature also. The endosomes present in the nucleus are vacuolated but less disintegrated than those of late prophase. The large endosome that appears to be lying partly outside the membrane is in a wrinkle and is included in the nucleus. No centrioles were observed. The oblique angle of the equatorial plate to the longitudinal axis of the animal is typical of sections made of individuals in metaphase, or, in some cases, late prophase. Ivanič (1934) found a similar condition in two species of *Euglypha*. This, he considered, indicated a transition stage from longitudial to transverse division. Metaphase is of very short duration, lasting only about 5 minutes.

Anaphase.—No stages of early anaphase showing actual splitting of the equatorial plate were secured. Fig. 6-A shows a section of an animal in anaphase shortly after the chromosomes had separated. The cytoplasm is considerably contracted in the new shell, leaving it partially empty. In contrast to most previous stages, the cytoplasm is almost free from vacuoles. This individual had a large number of the dark staining bodies in the area surrounding the nucleus. The nucleus has now taken on an elongated shape, but its axis continues to be oblique. The nuclear membrane, while thin and slightly twisted, remains unbroken. The chromosomes have separated and moved poleward on the spindle, as can be seen in Fig. 6-B. Like the metaphase, these chromosomes have granules associated with them on the spindle fibers, though in general they are more clearly defined. Eight chromosomes can be made out on each side of the split plate. The endosomes have disappeared by this stage and will not be seen until the nucleus is reorganized. The role of the endosomes in mitosis of these animals is not clear. It does not seem likely they have any chromatin and probably consist largely of plastin material. Anaphase occupies a shorter time even than metaphase, certainly less than 5 minutes.

Telophase.—Shortly after the nucleus divides within the fundus of the old shell, there is a movement of the cytoplasm back into the new one so that sections made at this time are somewhat like the prophases. Fig. 7-A is a drawing of two animals just after the nuclei have separated. The nucelus of the parent individual is clearly seen, while that of the daughter does not show in this section. A number of the dark staining bodies associated with the cytoplasm around the nucleus can be seen in the new animal. Evidently they were carried over into the newly formed shell by the same movement of cytoplasm that separated the nuclei. Some of these bodies can be seen in the area connecting the two animals.

No sections of this stage showed the nucleus greatly elongated, and from the material at hand it appears that marked nuclear elongation does not occur. Fig. 7-B shows one of two nuclei which were near each other in the cytoplasm of the old shell and had evidently just separated. This nucleus is very small, with the chromosomes still in a form like the split plate seen in the anaphase, but less granular and more distinct. The spindle has entirely disappeared. By the time the nuclei have separated into the two animals, the chromosomes lose their identity and form a mass of granular material in the center of the nucleus. This is shown in Fig. 7-C. The nucleus has enlarged and around the periphery has appeared a rim of dark material which later forms the endosomes. At about this stage a flowing back and forth of the cytoplasm begins; the movement taking place between the two animals as they lie with their shells mouth to mouth. For the next 10 to 15 minutes this movement continues and ends only when the cytoplasm is divided between the two individuals. Pateff (1926) describes a similar cytoplasmic movement in *Difflugia mammillaris*. By this time the nuclei are in the reconstruction stage, as shown in Fig. 8-A. The cytoplasm is now concentrated in the fundus of both shells, fairly evenly divided as to volume and the number of the dark staining bodies in each individual. Details of the nucleus are shown in Fig. 8-B. Besides having enlarged considerably, the nucleus has regained its spherical shape. The chromatin granules are scattered over the nucleus on a net and are still large. The endosomes have reappeared around the inside of the nuclear membrane, though yet small in size. With the exception of the granules and the thread-like net, the reconstruction nuclei are much like the resting form. Soon after the separation of the cytoplasm the two animals will form pseudopods and move apart, thus ending the process. The duration of the telophase was between 15 and 25 minutes on the average, with somewhat more variation than the other stages.

The total time for mitosis averaged 1 hour and 20 minutes. The shortest time for the process was 1 hour and 2 minutes, the longest 1 hour and 40 minutes.

DISCUSSION

The similarity between division in *Pontigulasia vas* and that described for related forms makes a detailed comparison unnecessary. There are, however, some points worthy of discussion.

In *Difflugia mammillaris* and *D. pyriformis*, Pateff (1926) found that the granules and the net of early prophase arose from materials of the outer nucleus. The net and the granules of the resting nucleus break down before the prophase structures appear. In *Pontigulasia* there is some evidence that the net of lightly staining material scattered over the resting nucleus becomes the more definite structures of the prophases.

As has been mentioned before, the role of the endosomes in *Pontigulasia* during mitosis remains in doubt. The fact that they are present after the equatorial plate has been formed, and that they begin to reappear while the chromatin is still concentrated in the center of the telophase nucleus, supports the view that they have no important part in mitosis as far as the chromatin and the net

are concerned. It was because of the uncertain part played by these nuclear bodies that the term endosome has been used (Calkins, 1933).

The small number and definite outlines of the chromosomes in *Pontigulasia vas* are different from the condition described for the two species of *Difflugia* by Pateff (1926). The chromosomes of *Pontigulasia* are formed by the concentration of the chromatin granules of the early prophases. By metaphase the chromosomes have been formed, but they are granular in structure and they have many granules with them on the spindle. Though sections showing splitting of the entire metaphase plate were not secured, there is some evidence that a longitudinal division of the chromosomes occurs. This is shown in Fig. 5-B, where one or two of the chromosomes appear to be splitting in this way. In the anaphase some pairing may be seen, and this too would seem to support a similar view. The granules on the spindle fibers present another problem, but there is little or no evidence to indicate what happens to them. In early telophase the chromosomes are clearest and appear least granular. Evidently the granules associated with the anaphase chromosomes have now fused with them, or less likely, they disappeared with the spindle fibers. As far as could be determined, the nuclear membrane remains unbroken during the whole process of division.

The time intervals given for the different stages are open to some question, because it proved almost impossible to control the temperature during division. Low temperatures tended to retard the process, but as a whole the optimum temperature was well below that reported for *Amoeba proteus* by Chalkley and Daniel (1933) and Liesche (1938) or for *Amoeba dubia* by Dawson, Kessler and Silberstein (1935).

During the course of this work a few large cultures of *Pontigulasia* were kept under observation for two to three months at a time. If these cultures were allowed to get in poor condition due to overcrowding, lack of food, or failure to change the water occasionally, a kind of plasmogamous union took place between the animals. Usually this took place in pairs, but occasionally a number of individuals would associate together. On investigation this fusion was found to be a cytoplasmic one only. No spores or gametes were noted. Inactive stages were seen but these were of a temporary nature, the mouth of the shell being filled with a plug of mucous or detritus material, and without the formation of a cyst wall.

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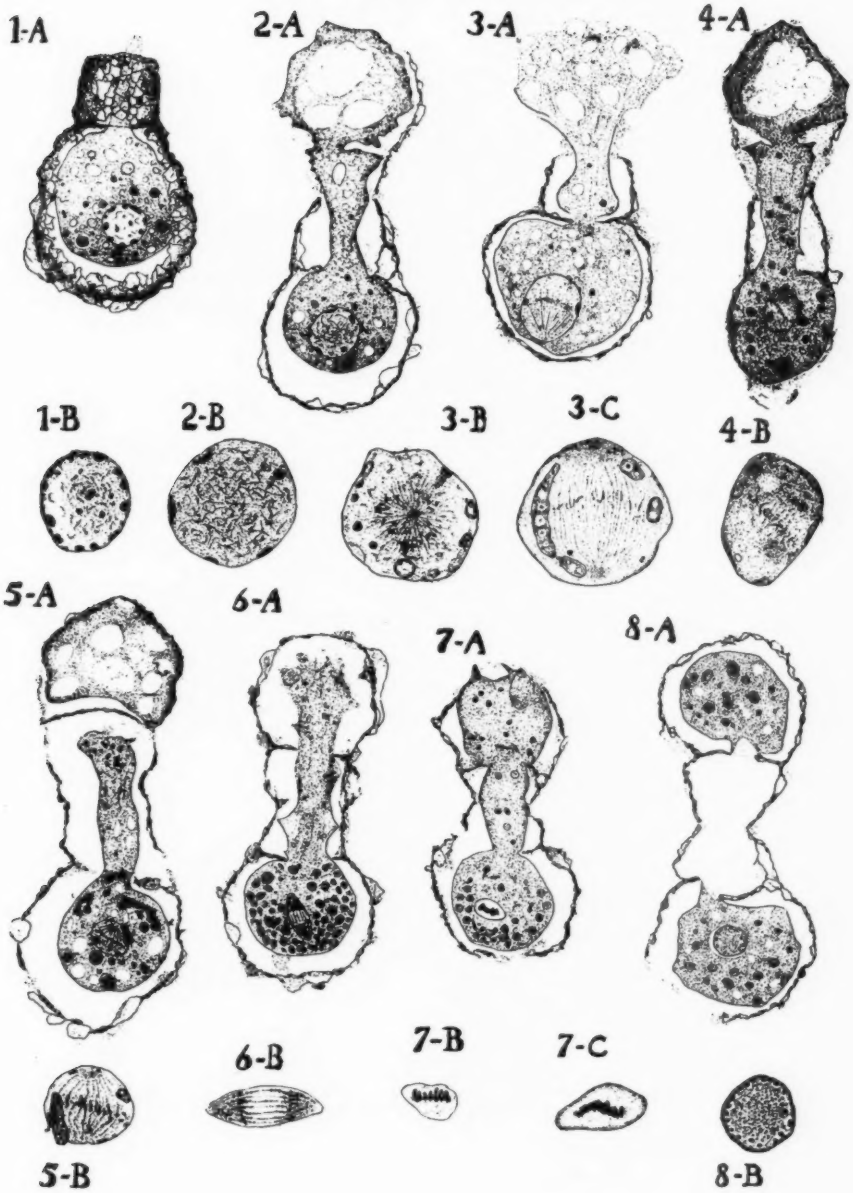
EXPLANATION OF PLATE 1

All figures, with the exception of 1-A, were drawn with the aid of a camera lucida from sectioned material.

Fig. 1-A. Diagrammatic drawing of *Pontigulasia vas*. Part of the shell has been omitted to show internal structure. × 400.
B. Section of a resting nucleus. × 800.
Fig. 2-A. Early prophase. Formation of the new shell is well advanced. × 400.
B. Details of the nucleus shown in Fig. 2-A. × 800.
Fig. 3-A. Middle prophase, but shell formation has hardly started. × 400.
B. Nucleus in middle prophase. × 800.
C. Nucleus in middle prophase. × 800.
Fig. 4-A. Late prophase. The new shell is almost complete. Note the constriction in the cytoplasmic mass; this marks the position of the diaphragm. × 400.
B. Details of the nucleus. × 800.
Fig. 5-A. Section of an animal in metaphase. × 400.
B. Details of the nucleus. × 800.
Fig. 6-A. Anaphase. The outlines of both shells are almost complete. × 400.
B. An enlarged view of the nucleus of Fig. 6-A. × 800.
Fig. 7-A. Telophase. Only one nucleus appeared in this section. × 400.
B. Details of a nucleus in early telophase. × 800.
C. Late telophase. × 800.
Fig. 8-A. A section of two animals shortly before separation. × 400.
B. Details of the reconstruction nucleus of Fig. 8-A. × 800.

PLATE 1



THE EFFECT OF ADRENOCORTICOTROPIC HORMONE ON THE INTERRENALS (CORTICAL TISSUE) OF TRITURUS TOROSUS

By Claude A. Villee, Jr.

The anterior lobe of the hypophysis is known to produce many different hormones (Evans, 1935; Collip, 1934a, 1934b), among which are the growth-promoting, gonadotropic, lactogenic, thyrotropic, diabetogenic, ketogenic, and adrenocorticotropic.

The first connection between the pituitary and the adrenal was noted by Brown (1920) who showed that extirpation of the thyroid and pituitary anlagen in *Rana pipiens* produced atrophy of the interrenal tissue. Several observers have reported that hypertrophy of the adrenal cortex follows administration of anterior pituitary extracts. Evans (1924) noted that the adrenal cortex was sometimes hypertrophied in his hyperpituitary giant rats. Reiss and Langendorff (1929) found enlargement of the adrenal in one of their dogs treated with a pituitary extract. Hypertrophy of the adrenal cortex following treatment with anterior pituitary extract was observed by Emery and Atwell (1933) in albino rats. Anselmino, Hoffman, and Herold (1933, 1934) made a very specific extract of the anterior pituitary which produced hyperplasia and hypertrophy of the adrenal cortex of female mice. Fat inclusions, which are normally found only in the zona fasciculata, were found in all three layers of the adrenal cortex after treatment with the extract. The glomerular zone was found to be twice as thick as normal. They found that a young male mouse was most sensitive to stimulation by the extract. Houssay, Biasotti, Mazzocco, and Sammartine (1933) also noted hypertrophy of the adrenal cortex in dogs after treatment with anterior pituitary extract. Loeser (1933) found hypertrophy of the adrenal after administration of thyrotropic extract to normal animals but not in thyroidectomized animals. Emery and Winter (1934) using anterior pituitary extracts, and McQueen-Williams (1934) using pituitary implants, failed to obtain hypertrophy of the adrenal cortex in thyroid-parathyroidectomized rats. Winter and Emery (1936) found that thyroidectomy does not affect the degree of compensatory hypertrophy which normally follows the removal of one adrenal. Atwell (1935) using a purified adrenocorticotropic extract of Collip, Anderson, and Thompson (1933) produced marked hypertrophy of the adrenal cortex and an increase of the osmiophilic content of hypophysectomized frogs. Atwell (1937) administered adrenocorticotropic extract to tadpoles of *Rana pipiens* and *Rana sylvatica* which had been hypophysectomized and thyroidectomized and produced hypertrophy of the atrophic adrenal glands and great increase in their osmiophilic content without any effect on the thyroid, showing that the action of the adrenocorticotropic hormone is not mediated through the action of the thyroid. Moon (1937) used a purified extract of adrenocorticotropic hormone to set up a method of biological assay of the extract. He obtained restoration of the cytoplasm of the cortical cells of the adrenals of hypophysectomized rats. The nuclei were no longer pycnotic and there was an increase in

the fat content of these cortical cells following the administration of an extract very similar to the one used in this study.

MATERIALS AND METHODS

Twenty adult *Triturus torosus*, each at least two or three years old, were collected from Wildcat Canyon, Contra Costa County, California. An extract of adrenocorticotropic hormone "containing some lactogenic and other residues as impurities" was obtained from Dr. Lyons of the Department of Anatomy, University of California. It was labeled L248I and was prepared according to the method devised by Lyons (1937). It contained 2.5 mg./cc. solid (active) material. This was injected subcutaneously into the lymph spaces beneath the chin and in the groin and axilla of the experimental animals.

The newts were kept in separate refrigerator dishes 5″ x 5″ x 2½″, in which water was placed and changed frequently. They were fed ground beef, liver, and mealworms.

The cortical tissue of the adrenal in Triturus is scattered in discrete patches along the branches of the renal arteries and veins on the ventromesial surface of the mesonephroi. It appears macroscopically as small orange-yellow clusters of cells around the blood vessels, especially frequent near their points of branching. There are at least forty such clusters of interrenal cells along the ventral side of the mesonephroi and several other clusters more anterior, along the course of the posterior vena cava.

In analyzing the effect of the injections of the extract, the entire mesonephros was removed together with the interrenal patches on its ventromesial surface and fixed in ten per cent formol for several days. The tissue was then washed in water and cut in frontal sections ten micra thick using the freezing microtome. These sections were dipped in 70 per cent alcohol momentarily, stained in Scharlach R ten minutes, placed in 70 per cent alcohol again for about ten seconds and then transferred to water, where the surface tension effect helped to spread them nicely. The sections were mounted in glycerine for study, and the cover slips rimmed with Euparal. The adrenals appeared as islands of cells containing droplets of lipoid material which stained red with Scharlach R in the mass of mesonephric tubules.

EXPERIMENTAL OBSERVATIONS

Fifteen of the twenty adult Triturus were used as experimental animals; the other five were reserved as controls. Table 1 gives the amount of extract given to each animal, the daily dose, the number of doses, and a comparison of the total dose with the body weight. All the animals in the experimental groups showed stimulation of the cortical tissue. The droplets of lipoid secretory material were both larger and more numerous in the experiment animals than in the controls. In general the amount of stimulation as estimated by a study of the comparative size and number of the lipoid droplets was proportional to the amount of hormone injected per gram body weight, although there are several deviations from this. There was no distinct evidence of hypertrophy of

the gland, but there was some evidence of hyperplasia in the animals most greatly stimulated.

Some very young Triturus had previously been injected with the extract in amounts varying from .03 to .15 cc. daily. It was found impractical to use animals of this age because they are apparently too sensitive to the extract, most of them dying within a short time even with the minimal dose. In addition, the adrenals are so small at this stage that in sectioning the mesonephros the few sections on which the adrenals were present were frequently lost.

The extract seemed to show some thyrotropic activity also, since the experimental animals moulted more often than did the controls. Moulting has been shown to be controlled by the thyroid in Triturus by Adams and Richards (1929).

Dr. Lyons has shown (unpublished data) that this extract will stimulate the adrenal cortex of twenty-eight day rats with a minimum threshold of about

TABLE 1

ANIMAL	DAILY DOSE, CC.	NUMBER OF DOSES	TOTAL DOSAGE		BODY WEIGHT, G.	MG./G.	AMOUNT OF STIMULATION
			In cc.	In mg.			
1	.10	11	1.10	2.75	12.5	.22	***
2	.10	6	.60	1.50	10.5	.14	***
3	.10	3	.30	.75	11.8	.06	**
4	.10	2	.20	.50	9.7	.05	*
5	.20	3	.60	1.50	13.8	.10	*
6	.20	3	.60	1.50	12.7	.12	**
7	.20	4	.80	2.00	11.4	.17	**
8	.20	4	.80	2.00	12.2	.16	**
9	.50	2	1.00	2.50	13.4	.18	***
10	.50	2	1.00	2.50	10.5	.23	***
11	.10	3	.30	.75	11.5	.06	*
12	.10	3	.30	.75	11.5	.06	*
13	.10	2	.20	.50	11.2	.04	*?
14	.20	2	.40	1.00	11.4	.09	*
15	.20	2	.40	1.00	10.9	.09	*

.30–.40 mg. hormone per gram body weight of the rat. This study showed that the minimum threshold for Triturus was about .06 mg. hormone per gram body weight.

SUMMARY

1. The interrenal tissue of *Triturus torosus* was shown to be stimulated by an extract of adrenocorticotropic hormone from the anterior lobe of the sheep pituitary.

2. Triturus adults are more sensitive to the extract than are twenty-eight day rats, the minimum threshold for Triturus being .06 mg. hormone per gram body weight, for rats, .30–.40 mg. hormone per gram body weight.

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RARE CYCLOPOID COPEPODS FROM WELLS IN NORTH CAROLINA

By Harry C. Yeatman

25 Text Figures

Early in November 1942, Mr. John M. Geary of the Orange-Person-Chatham District Health Department, Chapel Hill, N. C., brought to Dr. R. E. Coker in the Wilson Zoological Laboratory some copepods from a well in Chatham county. The copepods appeared to be of a form not previously known in this region and were given to me for further study. They proved to be examples of *Cyclops bicuspidatus navus* Herrick—a form we have long sought in this region. This find suggested exploration of a previously neglected habitat and Mr. Geary kindly took me to various wells in Chatham and Orange counties. I am greatly indebted to him for the opportunity to make this preliminary study conveniently as well as for assistance in the operations of collecting. I wish also to express appreciation to Dr. Coker for helpful suggestions in the preparation of this paper.

METHODS

Two weighted 12-inch plankton nets were used alternately at the wells and washed after each use to prevent mixing of collections. The net was simply lowered to the bottom, allowed to remain there a few seconds and then quickly raised. Microcrustacea entering the net would be washed into a bottle secured in the bottom of the net. The pH records are given as taken in the field with Hellige comparator.

All collections were made on November 25, 1942, except those made by Mr. Geary, November 4th and 5th, 1942, at the Wilkie Farm (station No. 4).

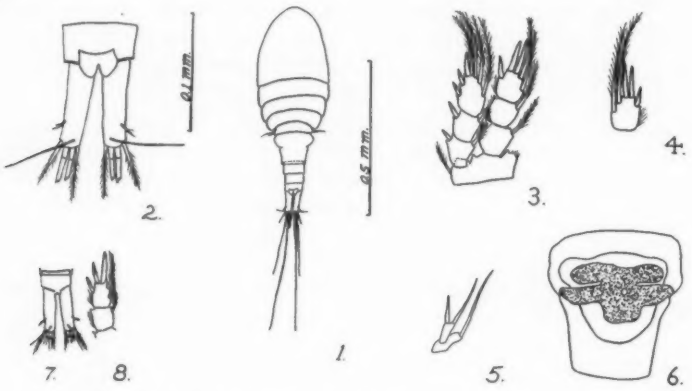
LOCATION AND DESCRIPTION OF WELLS

Station No. 1. Well at the home of Clarence Powell about 200 yards south of the Orange-Chatham county line, on the right side of U. S. 15-501 going towards Pittsboro, N. C. Top of well protected by wooden structure with a wooden door; water about 40 feet below surface of ground, but only a few feet in depth; pH 6.2. The rare *Cyclops crassicaudis brachycercus* Kiefer was found abundantly in this well. Hitherto it has been found in this region at only one place; see below, p. 30.

Station No. 2. Well at Lystra Church about 3 miles south of Orange-Chatham county line and about ½ mile east of U. S. 15-501. Well-top protected as in Station No. 1; water about 50 feet below surface of ground and only a few feet deep; pH 5.8. No copepods were taken by our nets.

Station No. 3. Well about 1.5 miles north of Farrington, N. C. Follow road past Lystra Church to T-junction; turn right to first house on left. Well top is of wood, as usual; water about 7 or 8 feet below surface of ground and only about 3 feet deep; pH 5.8. Copepods closely resembling the very rare *Cyclops nearcticus* Kiefer were fairly common.

Station No. 4. Well at farm of Bernice A. Wilkie about 12 miles east of Pittsboro, N. C., and at the end of a short dirt road north of U. S. 64. Well-top reinforced with brick and cement and enclosed in a screened porch; water about 42 feet below surface of ground and about 7 or 8 feet deep; pH 6.8; well dug in 1917. It was from this well that Mr. Geary's first collections were made, November 4 and 5; but the well had been chlorinated shortly before our collections were made, and we found no copepods. The original collections yielded many *Cyclops bicuspidatus navus* Herrick and several *Cyclops varicans rubellus* Lilljeborg.[1]



All figures are original camera lucida drawings unless otherwise stated.

FIGS. 1–8: *Cyclops nearcticus* Kiefer

Fig. 1. Adult female from Chatham County, North Carolina.
Fig. 2. Dorsal view of furcal rami and last abdominal segment.
Fig. 3. Fourth foot.
Fig. 4. Terminal segment of endopod of 4th foot.
Fig. 5. Fifth foot.
Fig. 6. Seminal receptacle of an anesthetized female.
(Figs. 2–6 drawn to the same scale.)
Fig. 7. Ventral view of furcal rami and last abdominal segment. After Kiefer, 1934.
Fig. 8. Second and third segments of endopod of fourth foot. After Kiefer, 1934.

Station No. 5. Well about ¼ mile south of Wilkie Farm (station No. 4) and on the same dirt road. Well with the usual wooden top; water about 25 feet below surface of ground and only a few feet deep; pH 6.2. *Cyclops crassicaudis brachycercus* Kiefer was found to be common. A few isopods (unidentified) were present.

Station No. 6. Well about 2 miles from U. S. 15-501 on the road from Pittsboro to Moncure, N. C. Well is on right side of road under an oak tree by an

[1] Three months later, collections in this well by Mr. Geary and Miss Ruth Naylon revealed the presence of copepods in this previously chlorinated well: two nauplii and one adult male identified provisionally as *C. bicuspidatus navus*.

unpainted frame house with two rows of boxwood. Top of well protected by the usual wooden structure, but with door of large-mesh wire-cloth instead of wood; water about 25 feet below surface of ground and only a few feet deep; pH 7.0. *Cyclops bicuspidatus navus* was very abundant. There were more copepods in this well than in any other.

Station No. 7. Well at Haywood School on U. S. no. 1, about $1\frac{1}{4}$ miles east of Moncure and 100 yards west of Haw River. Part of the wooden cover missing; well enclosed in a lattice house; a large terra cotta pipe extending deep into the well; water about 50 feet below surface of ground and at least 10 feet deep; pH 6.2. At the time of our collection, the well was condemned and locked because of the presence of *Bacillus coli*. Although this well contained much debris, including caterpillar feces, no copepods were present.

Station No. 8. Well across road from New Elam Christian Church about 2.5 miles north of U. S. no. 1. Well-top with large terra cotta pipe extending about 3 feet above ground; protected by a roof; water about 25 to 30 feet below surface of ground and only a few feet deep; pH 6.0. One male *Cyclops bicuspidatus navus* Herrick was found, but, since this copepod soon died and no others were collected, I believe this example had been retained in the net, despite washings, from our collections at station No. 6.

Station No. 9. Well at deserted frame house 2.7 miles from Wilson Zoological Laboratory on U. S. 15-501 by sign designating a curve. House and well on left side of road going towards Chapel Hill, N. C. Well with usual wooden top; water about 25 feet below surface of ground and only a few feet deep; pH 6.4. Several copepods closely resembling the very rare *Cyclops jeanneli* Chappuis were collected as were a few examples of the common (*agilis*) form of *Cyclops serrulatus* Fischer, with furca of moderate length.

DISTRIBUTION OF WELL-INHABITING COPEPODS

Only 3 (possibly 2) of 9 wells explored failed to yield cyclopoid copepods before chlorination. The wells may have been deficient in oxygen, but evidence points to a lack of introduction as the cause of the apparent absence of copepods. Copepods (*C. b. navus*) remained alive for weeks in laboratory finger bowls filled with water from wells Nos. 2 and 7, from which no copepods were obtained.

Adjacent wells do not necessarily contain the same species of copepods. Stations Nos. 4 and 5 are very close together, but their copepod fauna is different. More extensive well collections will have to be made before it can be determined if there are underground means by which copepods may be carried from well to well in this area. A small underground stream, but not likely a seepage through sand, would be able to transport cyclopoid copepods from well to well. Heavy rains might convey copepods from surface waters into wells located in low ground. Dirty water buckets, collecting nets, and water samplers might also innoculate wells with copepods, bacteria, and other organisms. On the other hand, most of the species collected in these wells are rare in America and, indeed, are very rarely found in surface streams, lakes, or ponds. Extensive

collections in the latter habitats in the vicinity of Chapel Hill has failed to yield *Cyclops nearcticus*, *Cyclops jeanneli*, or copepods closely resembling them. Kiefer (1934) described *C. nearcticus* from a single female sent by E. G. Hutchinson in 1931 from "Guilder Pond, Berkshires", Massachusetts, and Chappuis (1929) described *C. jeanneli* from examples taken from Morengo Cave in Indiana. These two species have not been reported since their original descriptions. Our records of *C. bicuspidatus navus* at stations Nos. 4 and 6 are the first records, to our knowledge, for this form in the southern part of the United States. It has been collected, however, in Minnesota, Illinois, Wisconsin, and possibly other northern states. *C. crassicaudis brachycercus* has been found only in a collection from New York (Kiefer, 1927), a few collections in Canada (Willey, 1929), and in collections from a particular, isolated rut in Fire Lane Road, Chapel Hill, N. C. Only *C. varicans rubellus* and *C. serrulatus*, which were found at stations Nos. 4 and 9, respectively, are commonly found in the numerous waters about Chapel Hill or have a known wide distribution in America.

DESCRIPTIONS OF THE RARER COPEPODS COLLECTED

Cyclops nearcticus Kiefer

Six examples in our collection were studied. Length of adult females varies from 0.50 to about 0.70 mm., the copepods being distinctly smaller than Kiefer's lone example of *C. nearcticus* which measured 0.80 mm. As with Kiefer's example, our copepods (fig. 1) resemble *Cyclops languidus* Sars. Furcal rami are about 4 times as long as broad (FW/FL, 0.250 to 0.297) and thus have the same proportions as *C. nearcticus* (cf. figs. 2 and 7). The outer lateral seta is attached at a point 69 to 74% of the distance from base to apex of the furcal ramus. Relative lengths of the terminal furcal setae, innermost to outermost, give ratios such as 1.04:11.0:5.40:1 and 1.40:10.40:5.50:1. The innermost terminal seta is thus slightly or substantially longer than the outermost terminal seta, although it is shorter than the furcal ramus (see fig. 2). Kiefer (1934) says this innermost seta is shorter than the outermost in *C. nearcticus* and shows this in his drawing (our fig. 7). Here, then, is a slight distinction between our copepods and the type of *C. nearcticus*.

First antennae are of 17 segments and extend to the posterior margin of the first body segment. The aesthete, or sensory club at the distal end of the 12th antennal segment, extends about to the middle of the 15th segment.

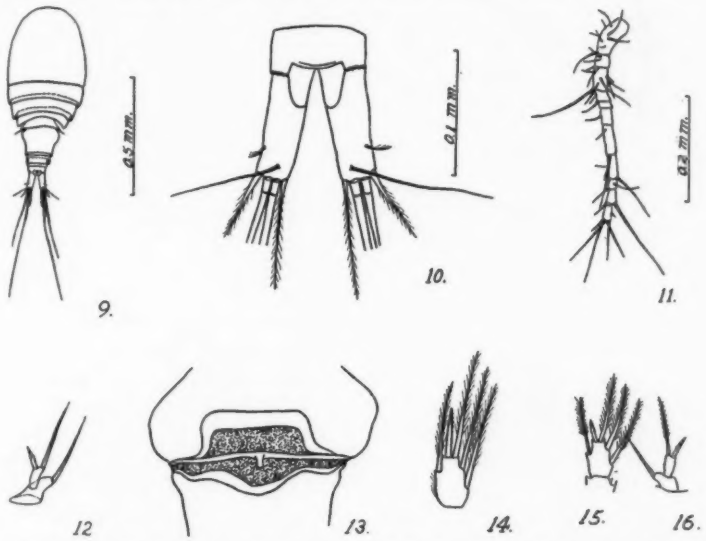
Swimming feet have the inner and outer rami of 3 segments. Spine formula of the terminal segments of the exopods is 2, 3, 3, 3. The complete armature of these feet is like that of *Cyclops bicuspidatus* Claus, except there is only one instead of two setae on the inner side of the second segment of the endopod of all four pairs of feet and a spine instead of a seta on the outer side of the terminal segment of the endopod of the fourth foot. Figure 8 is a copy of Kiefer's drawing of part of the fourth foot of *C. nearcticus*; it shows only one seta on the second segment of the endopod, as in our copepods (fig. 3).

The terminal segment of the endopod of the fourth foot is about 1.24 to 1.40 times as long as wide, thus being slightly stubbier than that of *C. nearcticus* (1.52 times as long as wide). The inner terminal spine is 1.14 to 1.23 times

longer than the segment and 1.14 to 1.16 times (about 1.42 times in *C. nearcticus*) longer than the outer terminal spine. As in *C. nearcticus* a short firm spine instead of a seta is on the outer side of this segment (figs. 3, 4 and 8).

The fifth foot (fig. 5) is much like that of *C. bicuspidatus* and other members of that subgroup. The seminal receptacle of an anesthetized female is shown in fig. 6.

Our examples are almost identical with *Cyclops nearcticus* as scantily described by Kiefer (1934) from a single specimen. The only real difference, innermost terminal furcal seta slightly longer instead of shorter than the outermost ter-



FIGS. 9–16: *Cyclops jeanneli* Chappuis and *Cyclops jeanneli putei*, n. subsp.

Fig. 9. Adult female *C. j. putei* from Orange County, N. C.
Fig. 10. Dorsal view of furcal rami and last abdominal segment of *C. j. putei*.
Fig. 11. First antenna of *C. j. putei*.
Fig. 12. Fifth foot of *C. j. putei*.
Fig. 13. Seminal receptacle of an anesthetized female of *C. j. putei*.
Fig. 14. Terminal segment of endopod of 4th foot of *C. j. putei*.
(Figs. 10 and 12–14 drawn to same scale.)
Fig. 15. Terminal segment of endopod of 4th foot of *C. jeanneli* (after Chappuis, 1929).
Fig. 16. Fifth foot of *C. jeanneli* (after Chappuis, 1929).

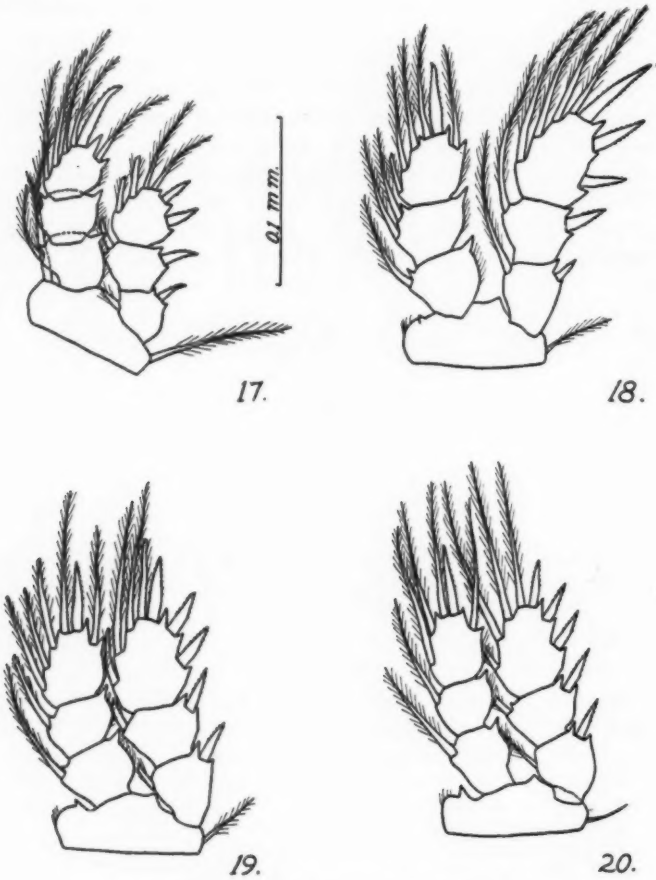
minal seta, is so slight that we identify our copepod as only a variant of *Cyclops nearcticus* Kiefer. The armature of the swimming feet, especially that of the fourth, separate this species from others with which it might be confused because of a similarity in the fifth foot.

Taken at station No. 3. Reported also from a single locality in Mass.

Cyclops jeanneli putei, n. subsp.

Three adult females and several males were examined but only the females are described here. Length of body of the females is 0.76 to 0.85 mm., not

including the terminal furcal setae, and thus our copepods are slightly smaller than Chappuis' *C. jeanneli*, which are about 0.9 mm. As in *C. jeanneli*, the genital segment is very broad (see fig. 9) and the anal operculum extends rather far posteriorly.



FIGS. 17–20: *Cyclops jeanneli putei*, n. subsp.
(All figures drawn to the same scale.)

Fig. 17. First foot. Note single seta on second segment of endopod.
Fig. 18. Second foot. Note two setae on second segment of endopod.
Fig. 19. Third foot. Note single seta on second segment of endopod.
Fig. 20. Fourth foot. Note single seta on second segment of endopod and replacement of long inner spine at terminal end of endopod by a long seta.

Furcal rami are about 3 times as long as broad (3.5 times as long as broad in *C. jeanneli*). Lateral seta is attached at a point 69 to 71% of the distance from base to apex of ramus. Relative lengths of the terminal furcal setae, innermost to outermost, give ratios such as 1.55:10.22:6.44:1 and 1.6:11.25:6.50:1 (about

2:12.5:7.5:1 in *C. jeanneli*). Here is a slight difference between our copepods and *C. jeanneli*, which has a relatively shorter outer terminal seta. The innermost terminal seta may be about the same length or slightly longer than the furcal ramus (fig. 10).

First antennae (fig. 11) are of 11 segments and extend to or slightly beyond the posterior border of the first body segment. First antennae of *C. jeanneli* are of 17 segments (Chappuis, 1929); there is a clear distinction between these two forms. The aesthete of the 7th segment extends slightly beyond the distal end of the 8th segment (fig. 11).

Inner and outer rami of the swimming feet are of three segments. Spine formula of the terminal segments of the exopods is 2, 3, 3, 3. Figures 17–20 show the four pair of swimming feet and their unusual armature. Note that there is only one seta on the inner side of the second segment of the endopod of the 1st, 3rd, and 4th feet, as in our *C. nearcticus* examples, but that the 2nd foot has two setae at this place. Chappuis (1929) did not give the armature of the feet of *C. jeanneli*, so that we cannot compare these parts.

Terminal segment of the endopod of the 4th foot is about 1.4 to 1.66 times (1.5 times in *C. jeanneli*) longer than broad. There is a long seta instead of a long, inner terminal spine in our examples as well as in *C. jeanneli* (see figs. 14, 20, and 15). No other copepods, ot our knowledge, have a seta here instead of a spine. This seta is 1.95 to 2.25 times longer than the segment and 1.91 to 2.25 times longer than the outer terminal spine. By our measurement of Chappuis' drawing (our fig. 15), this long seta is 2.07 times as long as the segment and 2.9 times longer than the outer spine. The fifth foot (fig. 12) is like that of *C. jeanneli* (fig. 16). The seminal receptacle (fig. 13) is very much like that of *C. crassicaudis*. There is a short tube extending from the outside opening to the chamber of the receptacle.

Since our copepods resemble *C. jeanneli* so closely in all respects except in the number of segments of the first antennae, and we are sure our examples are sexually mature, we can only consider them as representing a subspecies of *C. jeanneli*. A similar case is that of *C. b. lubbocki* which has first antennae with 14 segments and is generally considered a subspecies of *Cyclops bicuspidatus* with 17-segmented antenna. For our examples, we propose the subspecific name *putei*, referring to its discovery in a well. More than any other species that we have examined in the living state, *C. jeanneli putei* has the ability to adhere to the glass in a pipette when the water is forcibly expelled.

In general appearance *C. jeanneli* and *C. j. putei* closely resemble *C. crassicaudis* Sars; they differ from that species in having a long seta instead of a long spine at the inner distal end of the endopod of the 4th foot, 11 or 17 instead of 12 segments in the first antenna, and the innermost terminal furcal seta longer than the outermost instead of shorter.

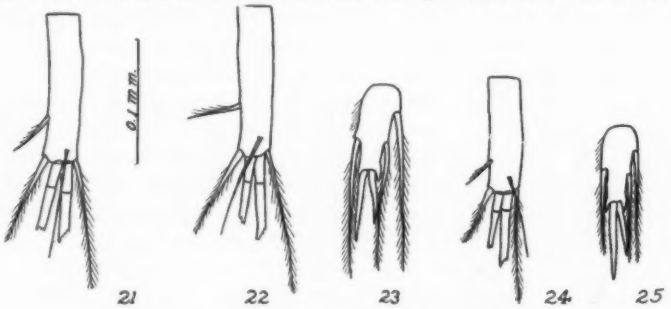
The species is known only from a cave in Indiana; the subspecies only from a well (No. 9) in Orange county, N. C.

Cyclops bicuspidatus navus Herrick

Since we have described examples of the *C. b. navus* collected at Wilkie farm (station No. 4) in a previous paper (Yeatman, 1943), we merely compare them now with the examples collected at station No. 6 near Moncure, N. C.

Adult female copepods from Moncure measured 0.90 to 1.00 mm. in length, not including the furcal setae, whereas the copepods from Wilkie farm averaged slightly larger but varied from 0.9 to 1.15 mm. As is readily apparent from the figures, the furcal rami of the former were much slimmer (FW/FL = 0.213 to 0.225 or between 4 and 5 times as long as broad—figs. 21, 24). In spite of this slenderness of the furcal rami of the Moncure examples, the small lateral seta averages no closer to the middle of the ramus than in the stubbier Wilkie examples. *Cyclops bicuspidatus* Claus, of which we still questionably consider *C. b. navus* a subspecies, has a very slender furcal ramus and the outer lateral seta at a point about the middle of the ramus. Attempts to mate the variants of *C. b. navus* with *C. bicuspidatus* and *C. bicuspidatus thomasi* S. A. Forbes will have to be made before we can know the specific status of these forms.

Quotient of length into width of the terminal segment of the endopod of the 4th foot is 0.340 to 0.370 in the Moncure examples and 0.330 to 0.375 in the



FIGS. 21–25: *Cyclops bicuspidatus navus* Herrick
(All figures drawn to the same scale.)

Figs. 21 & 22. Furcal rami; example from well near Moncure.
Fig. 23. Terminal segment of endopod of 4th foot; example from well near Moncure, N. C. Note that setae extend beyond distal end of the longer terminal spine.
Fig. 24. Furcal ramus; example from well on Wilkie Farm.
Fig. 25. Terminal segment of endopod of 4th foot; example from well on Wilkie Farm. Note that setae do not extend beyond distal end of the longer terminal spine.

Wilkie examples. Quotient of length of the segment into length of the inner of the two terminal spines is 0.831 to 0.850 in the Moncure examples and 0.937 to 1.01 in the Wilkie examples. Thus the inner spine in the Wilkie copepods is longer relative to length of the segment than it is in the Moncure examples. The setae of this segment extend far beyond the ends of the terminal spines in the Moncure examples (fig. 23), but such is not true in the Wilkie examples (fig. 25). The copepods from both collections were alike in all respects, other than those mentioned above. Thus the Moncure examples differ from the Wilkie examples in proportion of the furcal rami and in relative lengths of the setae and spines of the terminal segment of the endopod of the 4th swimming foot. Such differences seem to be genetic rather than environmental since they have remained constant in our cultures of the two lines. We have not attempted to mate copepods from the two lines, nor have we reared them under known experimental conditions.

Found at stations 4 and 6. The subspecies was known before only from North Central States.

All our *C. b. navus* were very white when taken from the wells, but when placed in fingerbowls with good light and an abundance of food (Euglena, Paramoecium, etc.), they become reddish brown in color because of the presence of oil droplets which seem to be stored food.

Cyclops varicans rubellus Lilljeborg taken at the Wilkie farm well is a fairly common species in surface waters: it is discussed in another paper (see Yeatman, 1943). *Cyclops crassicaudis brachycercus* Kiefer is treated in the same paper.

Cyclops serrulatus Fischer, taken at station No. 9, has extremely wide distribution.

SUMMARY

In Orange and Chatham counties, N. C., nine wells were explored with the plankton net hauled vertically, or in one case by drawing a bucket of water in the usual way.

Six yielded cyclopoid copepods and sometimes in considerable numbers.

Of 5 species taken: one is of almost universal distribution—*C. serrulatus* Fischer; another is a not uncommon species in surface waters of the region—*C. varicans rubellus* Lilljeborg; a third is a very rare species in America and, in this geographic region, has been known only from one temporary spring-fed pool—*C. crassicaudis*; two have never before been found in the region in spite of intensive searches of surface waters about Chapel Hill—*C. bicuspidatus navus*, known previously from North Central States and *C. nearcticus* Kiefer, hitherto known only from a single example taken in a pond in Massachusetts; the sixth is a new subspecies (if not a good species), clearly related to, but distinct from a copepod described by Chappuis from a cave in Indiana—*C. jeanneli putei*, n. subsp.

Only two species, *C. crassicaudis brachycercus* and *C. bicuspidatus navus*, were taken in more than one well and in each case the two wells investigated were many miles apart.

The results suggest that there may be a distinctive fauna of wells but throw no light on the origin of the fauna. The need for more general explorations of wells and subterranean waters is definitely indicated.

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NEW SPECIES OF NOWAKOWSKIELLA AND BLASTOCLADIA[1]

By Alma J. Whiffen

Plates 2–4

From one soil collection made at Terrell, Texas, two different fungi were obtained on grass leaf bait. One fungus proved to be a new species of *Nowakowskiella*, a genus of the Chytridiales, and the other a new species of *Blastocladia*, a genus of the Blastocladiales. The species of *Nowakowskiella* was isolated in unifungal culture by allowing several zoosporangia to discharge their spores in a drop of water, the zoospores then being transferred in a capillary pipette to a Petri dish containing a piece of boiled grass leaf and sterile water. A unifungal culture of the species of *Blastocladia* was established by dissecting from the grass leaf bait a number of mature resting bodies, which were dried and later induced to germinate by the addition of water. Since very few zoosporangia were produced by the *Blastocladia* and only a small number of new plants appeared in the cultures, material for study was obtained when needed by the germination of dried resting bodies.

At the present time four species of *Nowakowskiella* are recognized. Two of these species, *N. hemisphaerospora* (Shanor, 1942) and *N. profusum* (Karling, 1941), were described in the past two years. The other two species are *N. ramosa* (Butler, 1907) and *N. elegans* (Schroeter, 1897). Of these four species the present new species is most similar to *N. elegans* but differs from *N. elegans* in its more slender and less extensive rhizomycelium, in its more spherical apophysis, smaller zoosporangia, and smaller oil globules in the spores, and in its production of resting bodies. Because of the delicate character of the rhizomycelium, this new species of *Nowakowskiella* is named *N. delica*.

Nowakowskiella delica n. sp.

Rhizomycelium, intra- and extramatrical, much branched with numerous elongated swellings, which give rise to zoosporangia or resting bodies. Zoosporangia, terminal or intercalary, 12.5 x 15.0 μ to 22.5 x 28.8 μ, spherical, ovoid, or pyriform; with a single exit pore, usually laterally but often apically placed, operculate; apophysis variable in shape, 2.5 x 15.0 μ to 11.5 x 17.5 μ. Zoospores hyaline, spherical, 5.7–7.5 μ, posteriorly uniflagellate, flagellum 30 μ long, uniguttulate. Resting bodies, terminal or intercalary, usually intramatrical, ovoid to spherical, 10.0 x 15.0 μ to 26.2 x 29.9 μ, apophysate, hyaline, containing one to many oil globules. Germination of resting bodies not observed.

Saprophytic on a grass leaf placed in a soil collection made December 28, 1941, by Dr. J. N. Couch and Philip Couch at Terrell, Texas.

[1] This work was supported by a grant from the Penrose Fund of the American Philosophical Society. I wish to express my grateful appreciation to Dr. J. N. Couch for his interest in this study.

Extensive growth and branching of the rhizomycelium for a period of two or three days after inoculation of the grass leaf precedes the formation of the spindle-shaped swellings, which later may develop into zoosporangia or resting bodies. Frequently resting bodies are formed on the intramatrical portion of the rhizomycelium before any zoosporangia begin development. Maturation of the zoosporangia on the rhizomycelium is not simultaneous, for all stages of zoosporangial development may be seen along a line drawn from the point of inoculation to the tip of the youngest branch of the rhizomycelium (Fig. 11). As the zoosporangia mature and discharge their spores, the proximal portion of the rhizomycelium becomes vacuolate and inactive.

The origin of the apophysis is of interest in this species. After the enlargement of a terminal zoosporangial swelling is completed, a cross wall forms a little above the base of the swelling, so that the zoosporangium proper is subtended by an apophysis. In a non-apophysate species such as *N. hemisphaerospora* the cross wall, delimiting the zoosporangium from the rest of the rhizomycelium, develops at the base of the zoosporangial swelling. In *N. delica* because of the formation of the apophysis, only a portion of the protoplasm within the swelling is utilized in the development of the zoosporangium for the protoplasmic material thus cut off in the apophysis usually disintegrates. It should be noted that the apophysis is always cut off at the end of the swelling which is continuous with the older and dying portions of the rhizomycelium. Sometimes, however, the contents of the apophysis do not disintegrate as the zoosporangium matures (Fig. 9), whereupon the apophysis itself may enlarge, later being divided by a cross wall into zoosporangial and apophysate portions. In *N. delica* the usual condition in which the apophysis is cut off from the zoosporangium after the zoosporangial swelling has reached its maximal size is in contrast with the development described by Berdan (1941) for the apophysate species, *Cladochytrium hyalinum*. Here the zoosporangium and apophysis continue to increase in size after the formation of the wall delimiting the apophysis from the zoosporangium.

Nowakowskiella delica offers favorable material for following the stages in the development of the zoospores within the zoosporangium, because after the cutting off of the apophysis, the formation of the zoospores proceeds rapidly and is complete within ten or twelve hours. At about the time that the exit papilla makes its appearance, the refractive material in the zoosporangium is dispersed in small globules throughout the peripheral protoplasm which encloses a central vacuole. Upon the gradual disappearance of the central vacuole, the protoplasm throughout the zoosporangium appears to be cut up into numerous small masses, corresponding to the size of the zoospores (Fig. 3). Slowly the dispersed oil globules within each mass aggregate to form the single oil globule characteristic of the zoospores (Fig. 4, 5). Finally, preceding spore discharge, the protoplasm assumes an appearance of clearness in which it is impossible to determine with certainty the outlines of the individual zoospores (Fig. 6).

Resting bodies, like the zoosporangia, are apophysate (Fig. 13). The swelling which will give rise to a resting body cannot be differentiated from that which

will develop into a zoosporangium until after the formation of the apophysis. Then an incipient resting body can be recognized by the accumulation of numerous oil globules. As the resting body matures, these oil globules coalesce until as few as one, two, or three large oil globules remain. The wall of the resting body is only slightly thicker than that of the zoosporangium.

For *Cladochytrium elegans*, Schroeter (1897) created a new genus, *Nowakowskiella*, which was separated from *Cladochytrium* by the presence of an operculum in *Nowakowskiella* and the absence of an operculum in *Cladochytrium*. By use of the operculate or inoperculate character species are brought together in the same genus which are similar only in having or lacking the operculum and species, which are similar in every way except for the presence or absence of the operculum, are thus separated in two different genera. *Cladochytrium crassum* Hillegas (1941) resembles *Nowakowskiella profusum* Karling (1941) in the coarseness of the rhizomycelium, the lack of septations in the spindle-shaped swellings, the non-apophysate character of the zoosporangia, and the form of the resting bodies. *Cladochytrium hyalinum* Berdan (1941) is like *Nowakowskiella ramosa* Butler (1907) in the non-septate spindle-shaped swellings, the apophysate zoosporangia, and the formation of "pseudo-parenchyma" at the base of the resting body.

There is one character of the rhizomycelium in which all species of *Nowakowskiella*, as the genus is now constituted, agree and this character is the lack of septations in the spindle-shaped swellings of the rhizomycelium. Karling (1931) suggests that it is a matter of definition as to whether or not these spindle-shaped swellings in *Nowakowskiella* may be considered as true spindle organs. The spindle organs of *Cladochytrium replicatum*, which may be regarded as being typical, are cut off from the rest of the rhizomycelium by cross walls and seldom are directly transformed into zoosporangia or resting bodies. The swellings in the rhizomycelium of *Nowakowskiella* are continuous with the rest of the rhizomycelium and usually enlarge into zoosporangia or resting bodies. Neither *Cladochytrium hyalinum* nor *C. crassum* has typical septate spindle organs, a fact pointed out by Hillegas (1941) in comparing his species, *C. crassum*, with *C. tenue*. It is interesting that the two species in *Cladochytrium*, which differ from the other species in the genus in the lack of septations in the spindle-shaped swellings, are the two species that correspond so closely to the two species of *Nowakowskiella*, *N. profusum* and *N. ramosa*, as indicated above. The decision as to whether or not the septate or non-septate character of the spindle-shaped enlargements of the rhizomycelium is of more fundamental significance than the absence or presence of an operculum as a basis for separation of *Cladochytrium* and *Nowakowskiella*, must await further study of the species in the two genera and the discovery of new forms relating to these genera. Some students of the Chytridiales might prefer to remove from *Cladochytrium* the species which lack the septate spindle organs and to erect for them a new genus separated from *Nowakowskiella* by the absence of an operculum.

In Table 1 five species of *Nowakowskiella* and four of the better known species of *Cladochytrium* are compared as to the distribution among them of four characters: (1) nature of the spindle-shaped swellings, (2) the origin and form of the

resting bodies where known, (3) presence or absence of an operculum, (4) and presence or absence of an apophysis. Apophysate and non-apophysate species are found in both genera. Likewise there is variation in both genera as to the origin and form of the resting body.

The species of *Blastocladia* from Texas soil is remarkable for its smallness of size and simplicity of structure. The plant body, consisting of a cylindrical basal body and dichotomous branches, which lack pseudosepta and terminate in resting bodies or zoosporangia, is typically that of *Blastocladia*. In the smooth wall of the resting body and the presence of more than one exit papilla on the zoosporangium this species differs from all other described species of *Blastocladia*. Because of its small size, this species is given the name of *Blastocladia parva*.

TABLE 1

Comparison of species of Nowakowskiella and Cladochytrium

SPECIES	SPINDLE ORGANS	R.B.	OPERCULUM	APOPHYSIS
N. elegans	non-septate	unknown	present	present
N. ramosa	non-septate	with pseudo-parenchyma	present	present
N. profusum	non-septate	typical	present	absent
N. hemisphaerospora	non-septate	two-celled	present	absent
N. delica	non-septate	typical	present	present
C. replicatum	septate	typical	absent	absent
C. tenue	septate	unknown	absent	absent
C. hyalinum	non-septate	with pseudo-parenchyma	absent	present
C. crassum	non-septate	typical	absent	absent

Blastocladia parva n. sp.

Plant body cylindric, slender, 32 to 170 μ high, 12.5 to 50.0 μ wide; branching sub-dichotomous to dichotomous. Zoosporangia, irregular in shape, 40.0 x 65.6 μ to 41.3 x 90.2 μ, with one to six exit papillae. Zoospores from germinated resting bodies, posteriorly uniflagellate, with several small oil globules and a conspicuous food body, 5.6–6.1 μ x 2.8–3.2 μ. Resting bodies spherical, elliptic, ovoid, 36.8 x 41.0 μ to 35.2 x 77.1 μ; wall smooth, pale yellow in color; germination by cracking of outer wall and discharge of zoospores through one or two exit papillae.

Saprophytic in soil from Terrell, Texas, collected December 28, 1941, by Dr. J. N. Couch and Philip Couch.

Germination of resting bodies one month old or older, is readily induced, after a few days of drying, by the addition of water. The process of germination is the same as that described for *Blastocladia Pringsheimii* by Blackwell (1940) and for *Blastocladiella simplex* by Matthews (1937). No evidence of fusion of the zoospores from the resting bodies could be found. Zoospores from a large number of resting bodies were allowed to swim for an hour or longer before being killed with osmic acid and stained with gentian violet. All zoospores were then found to be uniflagellate.

Zoospores from germinated resting bodies develop into plants that are like those which previously bore the resting bodies. Development of the plant body is

rapid at room temperature, its growth and maturation being accomplished within four or five days. Branching of the germ tube gives rise to the intramatrical rhizoidal system while the elongating body of the spore constitutes the beginning of the basal body (Fig. 18, 19), which apically branches dichotomously or subdichotomously three to five times (Fig. 20). Upon the completion of the last dichotomy, elongation of the plant body ceases and protoplasm accumulates in the rapidly swelling tips of the branches. This enlarged apical portion of the branch is cut off by a cross wall and may develop into either a zoosporangium or a resting body (Fig. 21).

The development of the resting body is completed in about twenty-four hours (Fig. 25–28). As the wall of the resting body thickens, the oil globules break up into equal-sized particles and become evenly dispersed throughout the protoplasm. The thick outer wall of the resting body may be either widely separated or inseparable from the wall of the branch enclosing it.

During the course of this study zoosporangia were found but rarely. They developed on the same plant as the resting bodies (Fig. 33). Whether zoosporangia or resting bodies are produced in the greater abundance may be determined by conditions of the environment but the exact conditions which are necessary for the development of the zoosporangia are not known. At 24° and 25° C. only resting bodies were produced but in three cultures grown at 21°C. about a dozen zoosporangia were found.

Though more complex in form than any species of *Blastocladiella*, *Blastocladia parva* indicates a possible transition from the small tubular, unbranched body of *Blastocladiella simplex* to the large much branched plant body of such a species of *Blastocladia* as *B. ramosa*. In a culture of *Blastocladia parva* there are to be found plants with a single dichotomy, producing only two reproductive structures, plants with two dichotomies and four resting bodies, and finally plants with three to five successive dichotomies and eight to thirty-two resting bodies. The number of resting bodies, however, is variable and does not have a constant ratio with the number of dichotomies since not every branch tip matures into a resting body. Dichotomy in this case may be recognized as a more primitive type of branching than the sympodial, racemose, or cymose arrangements of branches, which are characteristic of the arbusculoid species of *Blastocladia*.

SUMMARY

Two new species are described. *Nowakowskiella delica* n. sp. is characterized by a delicate rhizomycelium with apophysate zoosporangia and resting bodies. Five species of *Nowakowskiella* and four species of *Cladochytrium* are discussed and compared on the basis of characteristics of the rhizomycelium.

Blastocladia delica n. sp. differs from all other described species of *Blastocladia* in the smooth wall of the resting body and the presence of more than one exit papilla on the zoosporangium. The possible derivation of this species from such a form as *Blastocladiella simplex* is discussed.

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EXPLANATION OF PLATES

PLATE 2

Nowakowskiella delica

Fig. 1. Zoospores. × 850.
Fig. 2. Spore discharge. × 850.
Figs. 3–6. Stages in development of a zoosporangium. × 850. Fig. 3—2:40 P.M. Fig. 4—2:50 P.M. Fig. 5—4:30 P.M. Fig. 6—9:00 P.M.
Fig. 7. Zoosporangial swelling. × 850.
Fig. 8. Zoosporangial swelling after the cutting off of the apophysis. × 850.
Fig. 9. An empty zoosporangium, subtended by an apophysis which is an incipient zoosporangium. × 850.
Fig. 10. Habit sketch of *N. elegans*. × 110.
Fig. 11. Habit sketch of *N. delica*. × 110.
Fig. 12. Intramatrical rhizomycelium. × 600.
Fig. 13. Resting bodies. × 850.

PLATE 3

Photomicrographs

Fig. 14. Rhizomycelium of *Cladochytrium replicatum* on agar. × 400. Note septate spindle organs. Photomicrograph by J. N. Couch.
Fig. 15. Rhizomycelium of *Nowakowskiella hemisphaerospora* on agar. × 150. Note non-apophysate zoosporangia and lack of septate spindle organs.
Fig. 16. Rhizomycelium of *Nowakowskiella elegans* on agar. × 430. Compare with the rhizomycelium of *C. replicatum*.

PLATE 4

Blastocladia parva

Fig. 17. Zoospore from resting sporangium. × 850.
Fig. 18. Germling, 12 hours old, from agar culture. × 300.
Fig. 19. Young thallus. 3:30 P.M. × 300.
Fig. 20. Thallus showing dichotomous mode of branching. 10:00 P.M. × 300.

Fig. 21. Thallus on which resting bodies are being cut off. 10:00 A.M. × 200.
Fig. 22. Thallus with matured resting bodies. 8:00 P.M. × 200.
Fig. 23. A much reduced thallus from an agar culture 24 hours old. × 300.
Fig. 24. A reduced thallus from a leaf culture, three days old. × 110.
Figs. 25–28. Stages in the development of two resting bodies. × 300. Fig. 25—10:15 A.M. Fig. 26—2:00 P.M. Fig. 27—7:30 P.M. Fig. 28—10:30 A.M.
Fig. 29. A resting body which appears to have developed from an incipient zoosporangium. × 530.
Fig. 30. A mature resting body. × 600.
Figs. 31–32. Two stages in the germination of a resting body. × 600.
Fig. 33. A thallus which produced both resting bodies and zoosporangia. × 110.
Fig. 34. Zoosporangium. × 300.
Fig. 35–36. Two empty zoosporangia. × 300.

PLATE 2

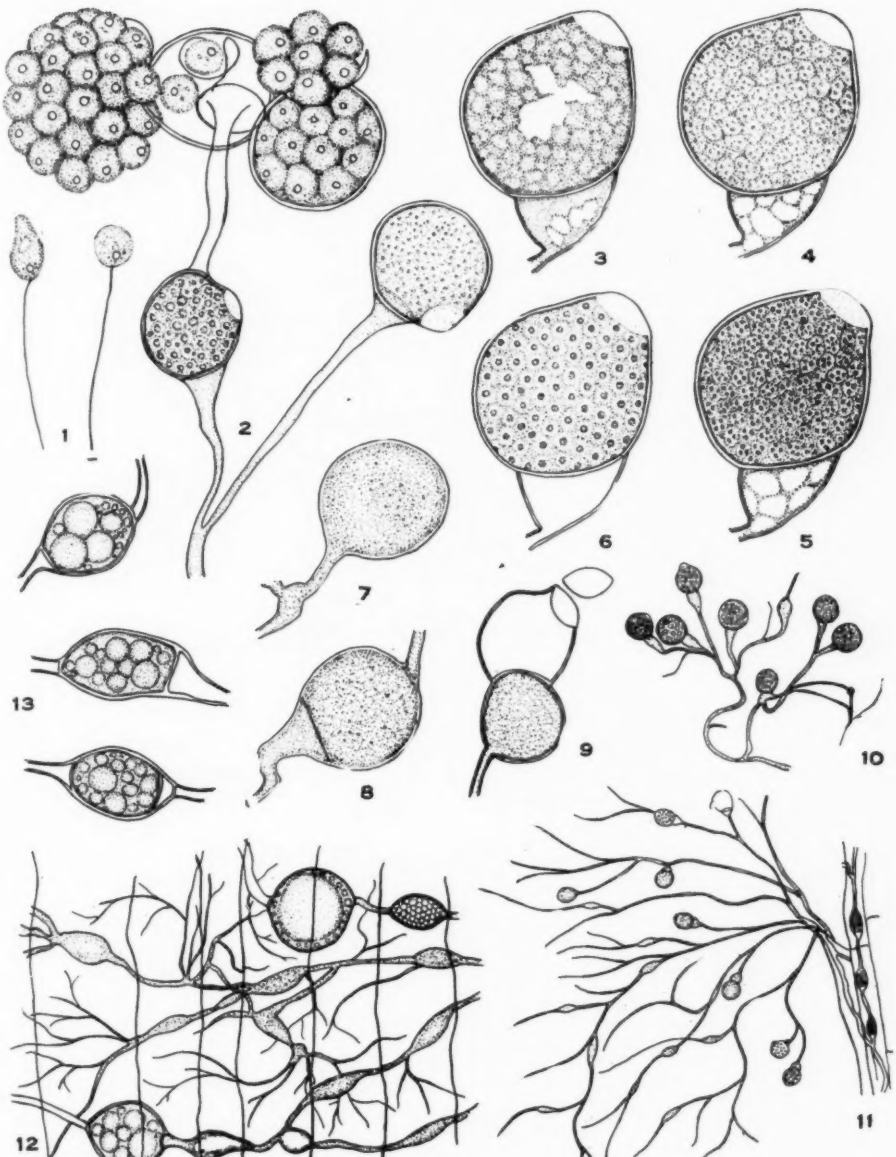


PLATE 3

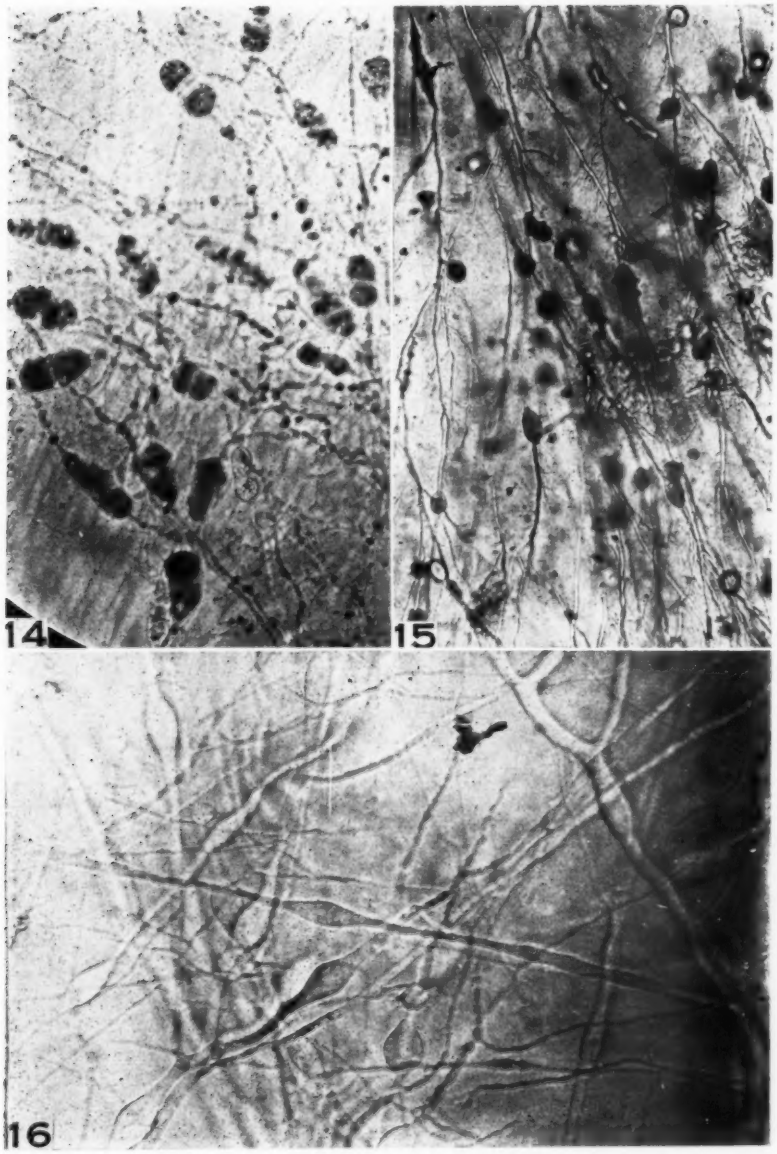
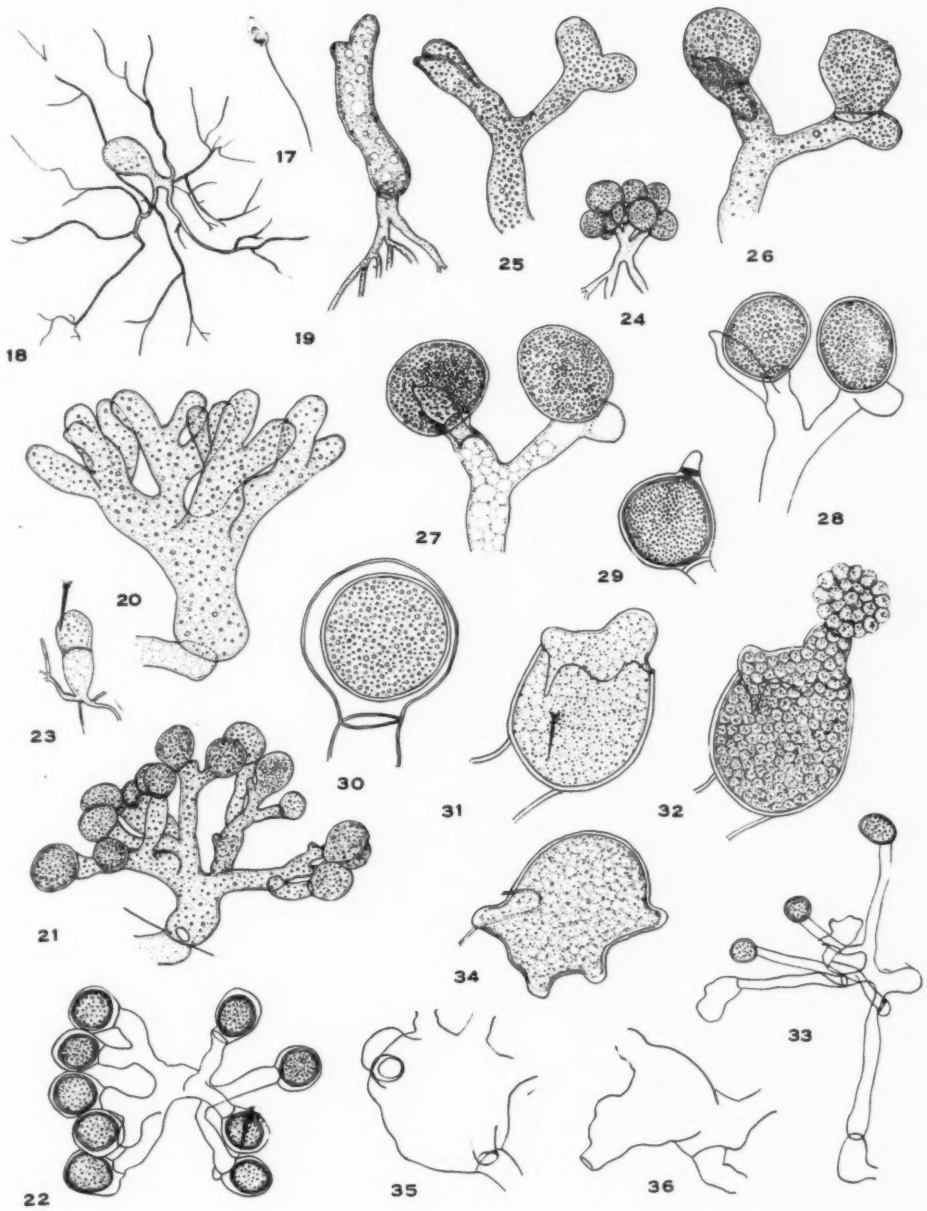


PLATE 4



MORPHOLOGY, CYTOLOGY, AND PARASITISM OF THEKOPSORA HYDRANGEAE

By Lindsay S. Olive

Plates 5–12

INTRODUCTION

Thekopsora hydrangeae Magn. is a heteroecious rust occurring abundantly in the mountains of our state on *Tsuga canadensis* and on several species of *Hydrangea*. Adams (1) proved by successfully inoculating leaves of *Hydrangea arborescens* with aeciospores from a rust resembling *Thekopsora vacciniorum* Karst. on *Tsuga canadensis* that the Canadian hemlock is the alternate host of *Thekopsora hydrangeae*.

The rust was pointed out to the writer by Professor W. C. Coker during a summer session at the Weyman Memorial Laboratory in Highlands, North Carolina. We have since found the rust in the vicinity of Asheville and Brevard in this state. Arthur (3) reports its pycnial and aecial phases on *Tsuga canadensis* (L.) Carr. in Indiana, Pennsylvania, Tennessee, and West Virginia, and on *T. caroliniana* Engelm. in North Carolina. The only alternate host for the uredinial and telial phases, as listed by Arthur, is *Hydrangea arborescens* L. We have found pycnia and aecia in abundance on *Tsuga canadensis* in this state, but no indications of it on *T. caroliniana*, although a considerable number of the latter species were examined carefully for the disease. In addition to finding uredinial and telial phases on *Hydrangea arborescens*, we have also found these stages abundantly on two other species of *Hydrangea*, *H. radiata* Walt. and *H. cinerea* Small.

Two other rusts, in addition to *Thekopsora hydrangeae*, were found on hemlocks in our area. *Thekopsora vacciniorum* was found sparingly on *Tsuga canadensis*, while its alternate hosts were *Azalea viscosa* L. var. *montana* Rehd., *A. arborescens* Pursh., *A. calendulacea* Michx., and *Cyanococcus similatus* Small. The latter three species have apparently not been reported among the hosts of this rust. One other rust occurring frequently on twigs and leaves of the Canadian hemlock is *Melampsora abieti-canadensis* (Farl.) C. A. Ludwig. The infections were found, upon microscopic examination, to contain numerous aecia. This rust causes the twigs to become yellowish and contorted.

It is expedient at this point to explain the nomenclature used in this paper. Adams (2) included all species belonging to the families *Coleosporiaceae*, *Melampsoraceae*, and *Uredinaceae*, with pycnia and aecia on gymnosperms, in one genus, *Peridermium*. The rusts included in this system are so varied that the present writer prefers to retain their former family and generic identities. Arthur (3) places in the genus *Pucciniastrum* forms with pycnia and aecia on various coniferous genera of the *Abieteae*, and with uredinia and indehiscent telia with vertically septate teliospores on seed plants. Magnus, in 1878, included the species in this latter group under two genera, *Phragmopsora* and *Thekopsora*,

respective of whether the teliospores are sub-epidermal or intra-epidermal. Pady (13), in a recent paper on teliospore development in this group, employs the generic name, *Thekopsora*, for species with intra-epidermal teliospores and retains the name *Pucciniastrum* for the species with sub-epidermal teliopores. The present writer prefers the usage of this last mentioned system of classification.

The chief purposes of this paper are to help clarify the position of this type of rust with relation to other groups and to present certain new findings in this group, or possibly for the rusts as a whole.

MATERIALS AND METHODS

Leaves of *Tsuga canadensis*, infected by *Thekopsora hydrangeae*, were collected on the grounds of the Weyman Memorial Laboratory in Highlands, North Carolina, during the month of June, 1941. Pycnia appeared during the earlier part of the month, while aecia were beginning to open by the middle of June. The uredinial stage was collected on the leaves of *Hydrangea arborescens*, *H. radiata*, and *H. cinerea* on the first of September and again in the middle of October. Fallen leaves with mature teliospores were collected from the ground around hydrangea bushes in October and December, 1941, and in March, 1942.

Diseased leaves of both the hemlock and hydrangea were fixed in formalin-acetic-alcohol and Flemming's weaker chromic-acetic-osmic acid solution. Small squares of the leaves were washed and embedded in paraffin. Sections were cut 6–7 μ thick.

Various staining procedures were carried out on sectioned material. A modification of Gram's Crystal Violet technique was quite useful in bringing out dividing nuclei against a clear cytoplasmic background. Heidenhain's hematoxylin was an indispensable nuclear stain and the most desirable one for bringing out nuclear details at all stages. Safranin and gentian violet were particularly useful in revealing certain important developmental details which were entirely lacking in material stained by the other two methods.

MACROSCOPIC OBSERVATIONS

The first external sign of the rust on hemlock leaves is the appearance of numerous little pycnia on the under surface. Frequently pycnia are also found on the upper surface, but never so abundantly as on the lower. They are rather inconspicuous and likely to be overlooked at first. With a hand lens one may observe that a sticky secretion is exuding from the ostiole of each mature pycnium. This sweet, sticky substance, which contains the pycniospores, presumably attracts insects, which spread the pycniospores from one pycnium to another.

Aecia appear at first as yellow dome-shaped protrusions on the lower surface of the leaf. They soon elongate and break open at the apex. The long, delicate peridia are quite conspicuous. The aecia are arranged in an orderly row along each side of the midrib, while pycnia are scattered in irregular fashion over the lower surface of the leaf (figs. 59 and 60). The infection causes the leaves to drop off early.

Uredinia appear later on in the season as small, bright orange-colored spots scattered profusely over the lower surface of hydrangea leaves. Uredospores continue to re-infect the hydrangea until the latter part of October. The telia of this rust are quite inconspicuous. If the upper epidermis of hydrangea leaves infected with old uredinia are examined in October, it may be seen to contain small brown patches or extensive brownish areas, which are groups of epidermal cells containing numerous teliospores. These spores remain viable and overwinter in the fallen leaves. All attempts to make the teliospores germinate precociously were unsuccessful. In the spring, if the leaves are moistened, basidia appear abundantly and impart a whitish bloom to the upper surface of the leaves.

MICROSCOPIC OBSERVATIONS

Early Infections on the Hemlock

The earliest infection stages observed were in sections of infected leaves in which pycnia had not yet appeared. These sections revealed the presence of numerous hyphae running through the leaf for considerable distances, ranging from the base of the leaf almost to the tip in some instances. These hyphae are much-branched and septate, with considerable intervals between septa. The branching hyphae extend from the upper to the lower surface of the leaf and their haustoria penetrate the host cells. These haustoria are most abundant after the aecia have appeared. They will be discussed more in detail later. The haustoria and cells of the intercellular mycelium are uninucleate.

Characteristic of this rust are the numerous hyphae which appear early beneath the cuticle and run for long distances parallel to the long axis of the leaf. These sub-cuticular hyphae are found beneath the cuticle of the lower and the upper epidermis, but are much more abundant in the former position.

It is apparent that the spread of rust hyphae is quite rapid once the leaf has become infected. This may be explained by the loose arrangement of the parenchyma cells in the hemlock leaf and by the facility with which the rust hyphae may run longitudinally beneath the cuticle (figs. 20 and 27). Hyphae pass with ease over the mid-vein from one side of the leaf to the other, but less readily beneath the mid-vein.

Development of the Pycnia

At irregular intervals in the sub-cuticular hyphae there appear areas in which the hyphae become more densely aggregated. Such an area is composed of more swollen and more septate hyphae than are found elsewhere at this stage. This is the early primordium of the pycnium (fig. 1). An area of basal cells is cut off from the hyphae, and each of these in turn gives rise to one or two pycniosporophores. There seem to be no paraphyses produced here, but flexuous hyphae appear, usually from the more central regions of the pycnium (fig. 2, f.h.). These may be readily distinguished by the fact that they are longer and more sinuous than the sporophores. Sometimes they are branched (fig. 9). In the

production of pycniospores, nuclear division takes place in the sporophore. A small spindle may be observed at this time. Figures 3 and 4 show the spindle at anaphase. These figures give evidence of the presence of a centrosome at each pole of the spindle. Figure 5 shows a telophase spindle; while the tip of the sporophore is beginning to constrict. In figure 6 the nuclear division is complete, and one of the two nuclei has passed into the constricting tip, which will be cut off as a pycniospore. This pycniospore is shed before the next one is produced. Pycniospores are ellipsoid, each with a large dense nucleus and a scanty supply of cytoplasm (fig. 7).

When the pycnium matures and begins to produce pycniospores, the pressure of the hyphae and spores causes the cuticle to rupture. Since the hyphae are longer near the center of the pycnium, more pressure is exerted upon the cuticle here than elsewhere. As a result, the cuticle is pushed up in a conical fashion and ruptures at its apex in an almost regular manner, so that the pycnium has a definite ostiole (fig. 2). It is through this ostiole that the pycniospores are extruded in a sticky secretion. Plate 12, fig. C, is a microphotograph of a mature sectioned pycnium.

There are some indications that pycniospores may fuse with flexuous hyphae, although no actual passage of a pycniospore nucleus into a flexuous hypha has been observed. Figure 8 shows what appears to be an empty pycniospore after fusion with such a hypha. Figure 9 is a group of flexuous hyphae, some of which show branching. One of the hyphae, only the tip of which is shown, has a group of pycniospores clustered about it.

Development of the Aecia

The first indications of the appearance of the aecial primordium come before the pycnia have matured. These primordia at first consist of small aggregations of intermingled hyphae, which appear at rather regular intervals in the wing of the leaf on either side of the mid-rib. They are located slightly below the mid-region of the wing (figs. 56 and 57). The primordium is simply a region in the leaf where the hyphae undergo a more rapid growth, branching, intertwining, and becoming more septate than the surrounding hyphae. The primordium is by nature of its origin continuous with and a part of the whole branching system of hyphae in the leaf, including sub-cuticular hyphae, hyphae leading to and giving rise to the pycnia, and the hyphae passing between the parenchyma cells throughout the leaf. Stomatal hyphae, which are also a part of this system and may be traced to the aecial primordia, will be discussed more in detail later.

It should be remembered that the rust mycelium in the hemlock is a haplomycelium, with one haploid nucleus in each cell (figs. 1 and 2); whereas, many of the sections show that a number of the hyphae leading to the aecial primordia have cells with more than one nucleus (fig. 20). Such cells are not truly multinucleate, however, since the extra nuclei are constantly moving through the hyphae towards the primordia. There is good evidence that these nuclei have come into the hyphae through hyphal fusions within the leaf, fusions between pycniospores and flexuous hyphae, or through fusions between pycniospores and stomatal hyphae.

Figures 10–19 are mainly illustrations of hyphal fusions and nuclear migrations found in the hyphae just outside and leading into aecial primordia. Nuclei may pass through small pores in the cross walls of a hypha (figs. 12–14), or they may pass from one hypha into another through a fusion pore (figs. 10, 11, and 19). Figures 15–17 show an interesting method of hyphal fusion often found at the edges of the primordium. In this case, a hypha containing a nucleus attempting to pass into the primordium puts out a lateral protuberance into which the nucleus passes (fig. 15). This protuberance then fuses with a nearby hypha leading into the primordium, and the nucleus passes into the other hypha (figs. 16 and 17). Figure 18 shows one of the many nuclear divisions occurring in the aecial primordium during its development.

In addition to the probability that pycniospores fuse with flexuous hyphae, it appears that pycniospore nuclei may also enter by way of the stomatal hyphae (figs. 22–25). Figures 22 and 24 show stomatal hyphae, each with two nuclei. In figure 22 one of the two nuclei is moving through the hypha, as is indicated by its elongate form. Around the swollen tip of the hypha can be seen a cluster of pycniospores, two of which seem to have lost their nuclei. No actual passage of a pycniospore nucleus into a stomatal hypha has been observed by the writer, but this phenomenon is believed to be of frequent occurrence. Stomatal hyphae are very abundant in diseased leaves. In figures 23 and 25, the swollen and darkly staining hyphal tips have been killed by the closing of the stomata upon them.

As the weft of hyphae composing the aecial primordium continues to grow in size, it becomes differentiated into two distinct regions (fig. 26). The outer area (towards the lower epidermis) is the plectenchyma, which consists of cells that increase in size and decrease in cytoplasmic content. The inner region is the developing hymenium and consists of cells that increase less in size, but more in cytoplasmic content (fig. 27). Eventually the cells of the plectenchyma, along with any host cells in this region, completely disintegrate. Plate 12, fig. A, is a microphotograph of the same aecial primordium illustrated by figure 27 in the drawings. This photograph shows the position of the primordium in the hemlock leaf and also three pycnia on the lower surface of the leaf. As may be seen here, the pycnia are quite near the primordium, and it is not at all difficult to find hyphae connecting the pycnia with the primordium. It is through such hyphae as these that the nuclear migrations already described were observed.

A study of the developing hymenial region of the primordium illustrated by figure 27 and Plate 12, fig. A, reveals some very interesting phenomena. A primordium at this stage shows various phases of cell fusion in the hymenium leading up to the formation of the aeciosporophores—the first truly binucleate cells to appear in the hemlock leaf. A slightly earlier stage than that shown in figure 27 reveals that the hyphae in the hymenial region have become arranged more or less parallel to one another, pointing from the base of the primordium towards the plectenchyma. Each cell is now enlarged and contains abundant cytoplasm and a single nucleus, which is quite expanded over its former size

(fig. 30). These rows of cells become rather closely packed together, so that a compact layer is formed at the base of the primordium.

During this growth, a number of hyphae running into the base of the primordium may be seen to contain nuclei which are still migrating into the developing hymenial region (figs. 28, 31–33). In figure 28, two nuclei are passing from cells of one hypha into a cell of another hypha through relatively large pores formed by cell fusions. Another nucleus is migrating through a cross wall. Figure 29 shows a nuclear division in one of the basal hyphae. Figures 31 and 32 show nuclei migrating into the hymenium through small pores in the cross walls. Figure 33 shows a nucleus passing from one basal hypha into another through a small fusion pore.

When prehymenial hyphae reach the stage shown in figure 30, cell fusions begin to take place. These fusions were always observed to occur between adjacent cells of adjacent hyphae, never between cells in the same hypha. Adams (2) calls these fusing cells gametes. The present writer, however does not consider them true gametes, since there is no nuclear fusion in the resultant fusion cell, but the two nuclei remain separate until fusion occurs in the teliospore on the alternate host. It is believed, nevertheless, that the nuclei of the two fusing cells do have opposite sexual attractions for one another and that all the nuclei in any pre-hymenial row of cells are of the same sex. Otherwise there is no apparent reason for the lack of fusions between cells in the same row. It appears likely that nuclei of different sexuality may begin to come into the aecial primordium from its very first appearance as a small group of intertwining hyphae, since these hyphae may be connected with pycnia of opposite sex, as is indicated by hyphal fusions already described for this stage. Other nuclei reach the primordium all during the process of its development by means of nuclear migrations, as already described.

Although nuclei of opposite sexual attractions are in constant association with one another in the developing primordium, they show no tendency towards pairing until cell fusions occur among the swollen pre-hymenial hyphae. Furthermore, it would appear that, in the course of development, the nuclei of different sexes become segregated into separate hyphae. The inclination for cell fusion to occur comes only after the cells have matured in size and cytoplasmic content to such an extent as is illustrated by figures 27 and 30.

In the aecial primordium illustrated by figure 27, cell fusions are in progress, and already a number of binucleate fusion cells have appeared. The width of the mature aecium is pretty well determined by the width of the primordium at this stage. Cell fusions begin towards the center of the primordium and proceed towards its periphery. Stages in cell fusion are illustrated by figures 34–37 and 42 and 43. Figure 34 shows a fusion cell shortly after fusion has begun. Fusion cells have been referred to by many investigators as "2-legged cells". In figure 34, however, it is a 4-legged cell. This character is entirely dependent upon the point at which the cells begin to fuse and the stage at which fusion is observed. As fusion proceeds, the fusion cell may lose altogether its 2-legged or 4-legged appearance and become a regular binucleate cell (figs. 42

and 43). Figures 35 and 36 show two binucleate 2-legged cells with fusion proceeding from the apex towards the base. Figure 37 shows a multiple fusion cell with four nuclei. These occur only rarely, but probably account for the aeciospores described by some investigators as containing more than two nuclei.

An interesting phenomenon following the production of fusion cells is the disintegration of all the potential fusion cells distal to them (figs. 35 and 36). These cells, which are termed pseudoplectenchyma, gelatinize and disintegrate, adding to the disintegrating plectenchyma of the young aecium (figure 27, ps.p.). Now, as is often the case, if fusions occur in the more distal cells of the pre-hymenial hyphae followed by, or simultaneous with cell fusions deeper in the pre-hymenium, the outer fusion cells and all the non-fused cells in the outer area gelatinize and add to the disintegrating plectenchyma. Evidently, this disintegration is tied up with the fact that all the food coming into the young aecium is brought in through the basal hyphae. The deeper cells, therefore, are the first to receive this food material, and when the deeper cells fuse they are stimulated to grow and become aeciosporophores, thus cutting off the food supply to the cells beyond them. Figure 42 shows two healthy fusion cells deeper within the pre-hymenium, while above them there are at least three fusion cells and several uninucleate cells degenerating. Figure 43 shows a row of young aeciosporophores shortly before they begin to produce spores. All the pseudoplectenchyma and plectenchyma cells are degenerating, thus clearing the way for spore production.

The first division of the aeciosporophore produces a peridial initial (fig. 44, p.i.). The peridial initial, in turn, divides to produce a peridial cell and a smaller intercalary cell. Figure 44 shows the beginning of nuclear division in the peridial initial, while nuclear division is in progress in the sporophore prior to the cutting off of the first aeciospore initial. During these developments the nuclei divide conjugately. Spindles are generally irregular and difficult to interpret at this time, although occasionally there are indications of the presence of definite chromosomes on the spindles.

Figure 45 shows the peridial initial already divided into a peridial cell (p.) and a small intercalary cell (i.p.), while the first aeciospore initial (a_1) has been cut off and nuclear division is proceeding within it. Figure 46 reveals that the aeciospore initial has divided to produce a young aeciospore (a_1) and a small intercalary cell (a.p.). A second aeciospore initial (a_2) has been formed and another nuclear division is in progress in the sporophore to produce the third, and so on. Further stages in the development of the peridium show an increase in size and a decrease in protoplasmic content of the cells. The cell walls become rough and more thickened, while nuclei and cytoplasm degenerate completely (figs. 47–50). In rare cases it appears that an intercalary cell (figs. 47 and 48) or even the first aeciospore (fig. 48) may take part in the formation of the peridium.

As the peridial cells and aeciospores continue to grow in size and number, the pressure ruptures the lower epidermis of the leaf, the only remaining obstacle in its way. The peridium now extends itself to a considerable distance beyond

the lower epidermis, while the pressure of the spores on the inside causes it to rupture at its tip, making an opening through which the spores are shed (figs. 59 and 60). This outer peridium is quite delicate and is usually sloughed off in the sectioning and staining treatment.

Clearer mitotic figures were observed in hymenia of open aecia (figs. 51–55). Figure 54 indicates the presence of polar radiations, but these may have been artifacts. The spindle in figure 55 has two definite centrosomes, and the chromatin has the semblance of being composed of definite chromosomes.

The peripheral sporophores in the mature aecium do not form aeciospores, but are given over to the production of rows of lateral peridial cells, which are continuous with the earlier formed peridial cap (figs. 61–63). Peridial initials are cut off from the sporophores exactly as described for the aeciospores. The peridial initial, however, becomes divided by an oblique wall which cuts off an angular intercalary cell. This cell soon disintegrates and is thrown out of the peridial chain; while the peridial cells elongate and their end walls come together and adhere to form a continuous chain.

When the peridial cell is finally mature, it is apparently lifeless (fig. 64). Cytoplasm and nuclei have almost completely disintegrated, while the inside wall is much thickened and crenulate. The outside wall of the cell is much thinner and only slightly roughened.

One interesting feature not yet discussed is the fate of the intercalary cell. As soon as this cell is cut off from the spore initial it begins to gelatinize (fig. 65). This is indicated by the fact that its cytoplasm stains more darkly with gentian violet than that of the other cells. The writer has found that, as a general rule, gelatinization is indicated by a special affinity of the gelatinizing parts for gentian violet. This criterion almost invariably holds true with the safranin-gentian violet technique, but rarely with gentian violet as used in Gram's technique. In the latter case, the gelatin is apparently removed by the bleaching agent, oxalic acid.

Most of the gelatinization in the aecium takes place from the time the intercalary cell is first cut off until it becomes the third or fourth intercalary cell in the spore chain. During the process, this gelatin which is liberated from the disintegrating intercalary cells becomes spread about between the spore chains and takes an intensely blue stain with gentian violet. In figure 65, the gelatin between the chains is indicated by very dark strippling. However, it is not granular in nature, but appears as a homogeneous substance. It may also be observed in this figure that the lower intercalary cells are becoming quite vacuolate as they proceed towards disintegration.

Since most of the process of gelatinization occurs in the upper part of the aecium, it is not at all surprising that this upper area has, in sectional view, a banded appearance, indicating the extent of the gelatin (Plate 12, figs. B and D). These blue-stained bands had been observed earlier by the writer in a study of aecia in several *Gymnosporangium* species, but no specific importance was attached to them at the time. If Plate 12, fig. B, is again referred to, there can be seen within the darkly banded area a row of deeply stained intercalary cells

extending across the entire width of the aecium. It is in this region that they are undergoing the greatest amount of gelatinization.

It is quite possible that the sporophores may add to the gelatin content of the aecium, as the darker-staining tips of the sporophores would indicate (fig. 65). The intercalary cells of the peridial chains undergo the same gelatinization process as described for intercalary cells of the aeciospores (fig. 63). Here the gelatin appears on the outside of the aecium.

During or following the gelatinization of its contents, the intercalary cell in the spore chain elongates to resemble a stalk cell (figs. 66 and 67). Eventually the vacuolate cytoplasm within the cell becomes almost entirely depleted, the nuclei disintegrate, and the cell wall breaks down (fig. 67). The aeciospore is then free to fall out of the aecium.

Obviously, one of the main functions of the gelatin in the aecium is that of protection. Firstly, it may preserve a layer of moisture around the more delicate cells of the spore chain. Secondly, it can provide a lubricant about these more delicate cells, so that, as they slide past one another during development of the closely packed spore chains, they are not injured. A third possibility, and one about which least is known, is that the developing spore chain may obtain a certain amount of nourishment from the surrounding medium. The most rapid growth in the development of the aeciospore takes place in this area, and the intercalary cell is often pretty close to disintegration before the spore is mature.

The aeciospore has an interesting development, particularly with regard to its wall structure. Progressive thickening of the spore wall may be observed in the spore chains shown in figure 65. This is not a thickening of the outermost wall, or *perispore*, but is a second wall, the *epispore*, which appears just within the perispore (fig. 68). Since the spore is expanding in size during deposition of the epispore from within, the outer part of this wall, which was laid down first and now undergoes little or no further growth, begins to split (fig. 69). At this stage, perispore can be distinguished from epispore. By the time the aeciospore attains its maximum size, the perispore has reached the limit of its elasticity. It becomes ruptured and is eventually sloughed off entirely, exposing the deeply crenulate epispore.

During this development, there is a spot at each end of the spore where the epispore is not laid down (figs. 68 and 69). These thin spots appear in the aeciospore wall opposite its two points of attachment to the intercalary cells. There is an elongate smooth area down one entire side of the spore where the epispore is very thin or entirely absent. Figure 71 shows this elongate thin area on one side of the spore in longitudinal section, while figure 72 shows it in cross section. These latter two figures show the appearance of the third and innermost spore wall, the *endospore*. It appears at about the time the aeciospore is shed and remains rather thin. Most of the figures show that the spore contains an abundant supply of vacuolate cytoplasm, the densest part of which is a peripheral layer just within the developing spore wall. Two moderately expanded nuclei with distinct nucleoli are located near the center of the cell. The thin

spots at each end of the spore are germ pores. It is not certain whether germination may occur through the lateral thin area, but the position of the germ tube on the aeciospore illustrated in figure 73 would indicate that this is probable.

Haustoria and Host-Parasite Relationships in the Hemlock

Examination of cells in infected hemlock leaves revealed the presence of numerous haustoria. These are typically elongate structures with short lateral branches or lobes (figs. 38–41). Each haustorium has a single nucleus (figs. 40–41) and is very narrow at the point where it penetrates the cell wall (figs. 38 and 39). The haustorium is frequently in contact with the host nucleus and definitely shows an affinity for starch grains in the host cell. Figures 38 and 40 show the haustorial lobes partly enveloping a number of starch grains, while most of the starch in the cell shown in figure 39 has been completely broken down and utilized by the parasite. This figure should be compared with the healthy cells in figure 21.

The extent of starch hydrolysis by the rust fungus is quite striking. Plate 12, fig. E, is a photograph of a sectioned hemlock leaf with an aecium on one side, while the last aecium has already been passed in sectioning on the other side. The less infected wing of the leaf shows numerous starch grains, staining purple with gentian violet, while the wing containing the aecium has conspicuously fewer starch grains.

The parasite causes a moderate degree of hypertrophy in the hemlock leaf, so that the host cells are somewhat larger than their normal size and the leaf as a whole becomes more thickened and irregular in outline (Plate 12, fig. A).

During the course of this study, the question arose as to why the aecia were so regularly spaced in a single row on either side of the mid-rib (fig. 59). The explanation is based upon the method of infection and upon spatial and food relationships. First, a re-examination of the figures will show that an aecium is located at about the widest portion of the wing and that there is hardly space for another aecium in the same section of the wing. Secondly, because of the facility with which infecting hyphae may spread rapidly through the leaf early in development, aecia may develop on both sides of the mid-rib and along the entire length of the leaf at about the same rate. Furthermore, the tendency is apparently towards the production of as many aecia as possible without interference in food relationships between them. Thus, the equal spacing in the leaf.

Infection of the Hydrangea and the Development of the Uredinium

Only a few stages in the initial infection of hydrangea leaves by the aeciospores have been observed. Aeciospores produce infection hyphae which enter through the stomata and then branch out within the leaf (figs. 73–75). These branching hyphae soon come into contact with the mesophyll cells and send haustoria into them (fig. 76). The hyphae are septate at irregular intervals and hyphal cells are binucleate. Likewise, the haustoria are binucleate. The haustorium is typically a pyriform or reniform swelling at the end of a narrow stalk, which is even more narrow at the point where it penetrates the host cell wall (figs. 77–80).

It arises from an intercellular hypha at a point where the latter is pressed closely against the host cell. In some of the older cells, where the plasma membrane had become warped in places, it could be seen that the haustorium does not penetrate the plasma membrane but invaginates it (figs. 77 and 79).

Vegetative hyphae in the hydrangea leaf are considerably larger than those in the hemlock leaf, but are never found in large numbers until the appearance of the uredinial primordia. At this time hyphae begin to aggregate in groups near the lower epidermis. These hyphae become more septate and give rise to upright and expanded binucleate cells which, at their tips, come into contact with the lower epidermis (fig. 81). The two nuclei in each of these cells are large and contain conspicuous nucleoli, while the cytoplasmic contents of the cell are quite dense and minutely vacuolate. This layer of binucleate cells constitutes the early hymenium of the uredinium. Its further development will be discussed more in detail. Figure 82 is a section through a young uredinium at a stage shortly after division has begun in the sporophores. As in the aecium, development proceeds from the center towards the outside. In fact, new sporophores are being added all along around the sides of the sorus.

As in the aecium, the first division of the sporophore produces a peridial initial (figs. 83 and 84). The inner cell is called the basal cell. During this development, nuclei divide conjugately, although the two spindles may not be at exactly the same stage of division (fig. 83). Polar radiations are occasionally observed. The peridial initial now divides to form a large peridial cell and a small intercalary cell (figs. 84–86). Figure 84 indicates the presence of centrosomes, as well as polar radiations, during nuclear division. The peridial cell quickly becomes vacuolate and its nuclei begin to degenerate (figs. 85–87, 92), while the intercalary cell degenerates completely.

The basal cell may now do one of two things. It may divide first to produce a uredospore initial (figs. 86 and 87), or it may produce a bud which in turn divides to form a uredospore and an intercalary cell (figs. 85, 91–93), the latter method being much the more common. In figure 86, a uredospore initial has been cut off from the basal cell, and in figure 87, nuclear division is apparently taking place in the initial. A bud, however, is being produced by the basal cell, and the small uredospore initial will probably disintegrate before division is completed. Figure 94 shows a uredospore initial just beginning to degenerate. In the material examined, few spores could be definitely ascertained to have come from these uredospore initials. Figure 88 shows a uredospore possibly derived in this manner.

The basal cell generally begins to bud just after the peridial initial divides (figure 85). Early disintegration of the peridial intercalary cell allows for this growth. The nuclei in the basal cell divide conjugately and send a pair of nuclei into the bud (fig. 91). This bud becomes separated from the basal cell by a cross wall (fig. 92), and a conjugate nuclear division occurs in the bud, which then divides to form a binucleate uredospore supported by a binucleate stalk cell (figs. 93 and 95). The stalk cell becomes elongate and, unlike the intercalary cell of the peridium, is persistent (figs. 93, 99 and 100).

There appears to be no definite number of buds which may be produced by a basal cell. One or several may be formed, depending upon the amount of cytoplasm that the basal cell is able to maintain.

After the peridial cells are produced, they elongate in a vertical plane, while their adjacent walls adhere to form one continuous peridium (fig. 104). As the protoplasmic contents of these cells degenerate, their walls, particularly the outer one, thicken (figs. 95, p, and 105, a.p.). Occasionally, during earlier development in the uredinium, a bud may force its way in between the peridial cells and enter directly into the formation of the peridium without dividing (fig. 98). Growth of the uredinium causes the epidermis of the leaf to rupture, and pressure from within the uredinium causes the peridium to burst open, allowing the spores to be discharged. Plate 12, fig. G, shows a mature uredinium just before opening. The peridium ruptures at the apex because there is more pressure at that point. Spores are shed through the opening, which is at first rather regular and ostiolar in appearance. There are, however, no especially modified ostiolar cells, as have been described by other investigators for related forms.

When the uredinium has completed its lateral expansion within the leaf, the last three or four rows of sporophores to appear at the sides of the sorus become peridial in nature, and their protoplasmic contents degenerate (fig. 105, l.p.). Their cell walls thicken somewhat, but never become as thick as those of the apical peridium (fig. 105, a.p.).

The mature uredospore, when discharged from the uredinium, has a very spiny wall and about 6 or 7 germ pores (fig. 103). The history of wall formation in the uredospore is quite interesting. Very early in the development of the spore, the cytoplasm just within the perispore wall assumes a regularly reticulated appearance. This effect is illustrated in figure 90, which is a surface view of the young spore. There are apparently a large number of little hemispherical hyaline areas in the cytoplasm which give it this effect, as can be seen in figure 89, a longitudinal section through the spore. The cytoplasm adjacent to these hyaline areas is denser than elsewhere in the cell. Although, with the staining methods used, no visible contents could be seen in the hyaline spots, they are evidently not vacant, since a slightly later stage will show that the spines are forming in them (fig. 96). These spines penetrate the perispore. While this is happening, the dense peripheral layer of cytoplasm lays down more cell wall material between the spines (fig. 97), and eventually the wall is evened out on the inside (fig. 101). There are, however, certain spots in which the epispore is not formed. These become the germ pores of the spore (figs. 101 and 103). The wall bearing the spines is called the epispore.

A third wall is now laid down just within the epispore. This is the endospore (fig. 102). It does not become as thick as the fore-mentioned wall. The perispore here, unlike that in the aeciospore, is persistent and remains closely applied to the spore as its outermost wall. The spines, moreover, appear to be emanating from this outer wall; whereas, actually they are penetrating it. This could be determined only in well-stained sections of mature uredospores, in which the perispore stains with gentian violet and the other two walls remain unstained (fig. 102).

Uredospores reinfect the hydrangea throughout the latter part of the summer. Their spines are well adapted for securing their attachment among the hairs on the lower surface of the leaf. The uredospore produces a germ tube which enters the stoma (figs. 106–109). Upon entering the stoma, the germ tube becomes lobed into a number of little swellings, or sub-stomatal vesicles. Nuclear division is rapid during their formation and each lobe is binucleate (fig. 109). The lobes now give rise to hyphae which penetrate the leaf in all directions (fig. 108). In figure 109, two germ tubes have entered the same stoma. The hyphae now go on to infect the leaf in the manner already described for aeciospore infections.

Development of the Teliospores in Hydrangea

So far, infection in the hydrangea leaf has been confined almost entirely to the spongy tissue. However, towards the latter part of the growing season, hyphae running along more or less horizontally beneath the palisade layer give rise to upright swollen hyphae that push up between the palisade cells to the upper epidermis (fig. 110). The vertical hypha receives a rich supply of cytoplasm and two nuclei, which become quite expanded and contain conspicuous nucleoli. Upon reaching the cell wall of an upper epidermal cell, the vertical hypha penetrates it with an apical papilla (fig. 111), which expands immediately upon entering the cell (fig. 112). The papilla evidently enters by dissolution of the cell wall, since there is no indication of pressure being exerted on the cell wall by its relatively blunt tip. The vertical hypha is much broader at its point of entry into a cell than is the haustorium.

As the globular swelling of the hypha within the epidermal cell continues, the two nuclei and most of the cytoplasm in the vertical hypha pass into it (figs. 112–114). In transit, the nuclei become drawn out into very fine threads as they pass through the constriction in the hypha. The nuclear membrane remains intact during the process and expands within the swelling, while decreasing in size in the lower part of the hypha. The chromatin in the lower portion of the nucleus streams around the periphery of the membrane and passes upwards through the constriction to redistribute itself in fine strands radiating about the periphery of the expanding membrane within the swelling (figs. 112 and 114). The binucleate swelling is the teliospore initial (fig. 115).

While the teliospore initial continues to expand, the two nuclei divide conjugately and the initial divides into two binucleate cells (figs. 116–120). Spindles in dividing teliospore initials are usually in a plane parallel with the leaf surface. Figures 116 and 117 show anaphase spindles with small centrosomes and fairly distinct chromosomes. The telophase in figure 118 shows four distinct, though abnormally expanded, chromosomes at each pole. Figure 119 reveals the presence of polar radiations at the spindle tips. In figure 120, spindle fibers can be made out between the reorganizing nuclei, and the vertical cross wall is being laid down across the path of these fibers. Apparently, the fibers take part in the formation of the cross wall.

Like the haustorium, the teliospore initial invaginates the plasma membrane and does not penetrate it. This is indicated in figure 121, where the plasma membrane is, in places, warped away from the wall of the teliospore initial.

In rare instances an initial may become a single-celled teliospore, but more often it becomes a teliospore with 2–8 cells, the most common number being 4. Several initials usually enter the same epidermal cell.

Each cell of a maturing teliospore contains abundant cytoplasm and two rather expanded nuclei with distinct nucleoli (fig. 122). The spore wall now begins to thicken, with the exception of a small germ pore at the top (fig. 124). Coincident with thickening of the wall is the fusion of the two nuclei in each cell (figs. 123–128). First, the chromatin in each nucleus condenses into a spireme-like structure, or possibly into several dense strands. The nuclear membrane now seems to break down entirely, and chromatin threads from the two nuclei begin to intermingle (figs. 124 and 125). The chromatin of both nuclei becomes redistributed and combined into a single large fusion nucleus (figs. 126–128). The fusion nucleus now produces a new nuclear membrane and contains a single nucleolus.

In figure 129 can be seen a multicellular teliospore derived from a single initial. One cell has been cut off by an oblique wall. The spore walls have thickened, and the nuclei are just ready to fuse. Plate 12, fig. F, is a photograph of a prepared section through a hydrangea leaf and shows a row of teliospores in the upper epidermis.

Sometimes teliospores are produced atypically in cells other than those of the upper epidermis. Figure 130 shows a teliospore in a lower epidermal cell. In this case there is no swollen vertical hypha on the outside, but a couple of empty hyphae only slightly larger than ordinary vegetative hyphae, may be seen. Figures 131 and 132 illustrate what happens when the upper epidermal cells are already crowded with developing teliospores and the epidermal cell walls have become thicker. The tardy initials have entered palisade cells, and the teliospores are developing there. The dark areas in the figures represent regions which stained excessively with gentian violet and probably indicate a gelatinization of the host cell. Figure 133 shows the margin of an old uredinium in which teliospores have formed. It is not known whether these spores were formed within the walls of the old uredinial cells or whether they represent converted uredinial cells.

A few supplementary data have been collected on teliospore development in the related species, *Thekopsora vacciniorum*. Teliospores were found abundantly in both the lower and the upper epidermal layers in leaves of its ericaceous hosts, although Arthur (3) reports them as being only hypophyllous. Nuclear divisions in teliospore initials were observed, and spindles were found to have distinct polar radiations, while the number of chromosomes was four (figs. 134 and 135). These data correspond well with the data obtained for *T. hydrangeae*. Figure 136 shows a teliospore in a lower epidermal cell prior to nuclear fusion. Note the relatively small size of the hypha which produced the teliospore initial.

Basidia

Hydrangea leaves containing teliospores were kept over-winter in a moist place in our cold room. All attempts to germinate the spores before spring were

unsuccessful. Teliospores began to germinate on May 27, after being placed in a moist chamber at room temperature. Regular 4-celled basidia were produced.

Upon germination, each teliospore cell begins to extrude its contents through the apical pore. The young promycelium penetrates the epidermal cell wall and cuticle, and the entire protoplasmic contents of the teliospore cell pass into it (fig. 137). The nucleus enters into prophase (fig. 137) and the first meiotic division takes place (fig. 138). The two nuclei produced may go through a short resting phase (fig. 139) but soon undergo the second meiotic division (figs. 140 and 141). Distinct chromosomes may be defined on some of the spindles.

These two divisions result in a 4-celled basidium, each cell of which produces a sterigma into which the cytoplasm and a single haploid nucleus pass (figs. 142 and 143). As the sporidium forms at the tip of the sterigma, nucleus and cytoplasm pass into it (fig. 145). The chromatin of the nucleus becomes more condensed and draws out into a very thin strand as it passes from the sterigma into the sporidium.

The nucleus, after having entered the sporidium, begins to divide (fig. 146). This division is completed before the sporidium is discharged, and one of the two nuclei almost immediately begins to degenerate (fig. 147). The sporidium is discharged in the binucleate condition (fig. 148), but one of the two nuclei continues to degenerate, while the other expands. Figure 144 shows a group of basidia drawn from live material. The sporidia are the spores which infect the hemlock.

DISCUSSION

Aside from a limited account by Dodge (8) on the uredinium of *Thekopsora hydrangeae*, practically no research has been done on this species of rust. Although several isolated works have been carried out on the various sori in the genus, no one has produced anything on the complete life history of any representative in the group, and there have been a number of questions on development in need of clarification. The present work clarifies some of these points and presents new ones to be considered.

The discovery of the production of a gelatinous layer in the aecium, chiefly by disintegration of the intercalary cells, appears to be new for the rusts. The intercalary cells, which eventually elongate and look like true stalks, simulate the very gelatinizable stalk cells of the teliospores of *Gymnosporangium*. The writer has also observed a similar process in *Gymnosporangium* aecia. Intercalary cells and stalk cells are homologous structures, since they are both modified spores.

Nuclear migrations into the base of the aecial primordium, as described in this paper, are similar to those described by Christman (6) for *Phragmidium speciosum* Fr. and *Caeoma nitens* S. In the primordia, he found a fusion of what he called equal "gametes." Blackman (5) describes for *Phragmidium violaceum* Wint. a migration of nuclei into the primordium through small pores in the cell walls. In this case, however, the migrating nucleus enters directly into the basal cell without cell fusion. This latter method of establishing the binucleate condition was not observed in *Thekopsora hydrangeae*.

The present writer has found that, although there are abundant nuclear migrations into the aecial primordium, no truly binucleate cells are found until fusion occurs between cells of adjacent rows of pre-hymenial hyphae. Such cells have been referred to by Adams (2), Christman (6), and others as gametes, but they are apparently not true gametes, since nuclear fusion does not occur in the resultant fusion cell. Arthur (4) refers to the hyphae in which fusion takes place as "gametophytic hyphae" and calls the cells which fuse "cells of the fertile part of the primordium." Gäumann and Dodge (9) and Colley (7) refer to these last-mentioned cells as "fertile cells." The present writer uses the term *pre-hymenial hyphae* in preference to Arthur's "gametophytic hyphae," since all of the hyphae in the hemlock leaf may be considered gametophytic. It seems probable that, in the development of the pre-hymenial hyphae, sexual segregation of nuclei may take place so that cells of adjacent hyphae contain nuclei of opposite sexual attraction. Fusions were never observed between two cells in the same hypha, but they did occur often at more than one level in the same pair of hyphae.

Dodge (6) believed that the spore initials in the uredinium of this rust were cut off directly from the basal cells without the budding process, and that uredospores were produced in a manner approaching the catenulate arrangement. The present writer finds that by far the majority of spores are produced by budding of the basal cell, and that the spores are not catenulate in arrangement. Dodge's contention that the intercalary cells in the uredinium are sacrificed to furnish food for the developing uredospores is interesting. The present study of the aecium of *Thekopsora hydrangeae* has indicated even more convincingly that such is probably the case in relationships between the aeciospore and its intercalary cell. In a paper following that of Dodge, Moss (12) indicates, in a brief discription of the uredinia of *Thekopsora vacciniorum*, that uredospores are formed mainly by budding of basal cells. This is in agreement with the findings given in the present paper.

Ludwig and Rees (11), Dodge (8), and Moss (12) have described the uredinial peridia of various species of *Pucciniastrum* as possessing especially modified ostiolar cells with much thickened walls and often with spines. In *Thekopsora hydrangeae*, the cells around the ostiole are neither spiny nor unduly thickened and are not different from the other peridial cells.

The two common types of uredinia are those with a peridium, characteristic of the lower groups of rusts, and those without a peridium, characteristic of the higher groups. The uredinium of *Thekopsora hydrangeae* is of the former type, but differs from the uredinia of its related species in having no especially modified ostiolar cells, and with more persistent cells supporting the uredospores. These latter cells, therefore, resemble the stalk cells of the non-peridial forms and are an advancement over the more ephemeral intercalary cells found in many peridial forms. Thus the uredinium of *T. hydrangeae* affords a transitional step in the progression from lower to higher forms.

Uredospores, as well as aeciospores, were found to possess three walls, which are of rather similar origin for both spores. Lohwag (10) also observed the three

walls in uredospores of *Uromyces Erythronii*. Sappin-Trouffy (14) illustrated this three-walled condition for various types of spores in several different rusts. Upon examination of the wall of a young aeciospore of *Peridermium Pini*, he reported the presence of polygonal thickenings from which the spines appear. These are undoubtedly identical with the spine-forming hyaline areas observed by the writer beneath the perispore in the uredospore of *T. hydrangeae*.

Pady (13) recently described teliospore development in various genera of the *Pucciniastreae*, including *Thekopsora vacciniorum*. The rather peculiar type of nuclear fusion observed by the present writer for *T. hydrangeae* was briefly touched upon by Pady in a study of *Pucciniastrum epilobii* (Pers.) Otth. and *Hyalopsora aspidiotus* (Pk.) Magn. He states that "At first the two nuclei are small and homogeneous, but as they move together the chromatin is heavily staining and each nucleus has an irregular outline. The fusion nucleus is often to be seen in the spireme stage. . . ." The present findings on *Thekopsora hydrangeae* also reveal that the nuclei are probably in the spireme stage before fusion and that the nuclear membranes are broken down, a new one appearing in the fusion nucleus.

From studies of dividing teliospore initials, it appears that the chromosome number for this rust is 4. This count could not be accurately made in the developing basidia, because of a limited amount of material showing meiotic divisions. Sappin-Trouffy (14), in a study of germinating teliospores of *Thekopsora areolata* Wallr., reports the chromosome number as 2. This number was repeatedly given by Sappin-Trouffy for most of the rusts studied by him, and it is probably the result of a misinterpretation of the characteristic bilobed mass of chromatin at each end of the telophase spindle.

SUMMARY

1. Pycnia of *Thekopsora hydrangeae* Magn. are subcuticular in origin, occurring in the spring on both surfaces of the leaves of *Tsuga canadensis*, but more commonly on the lower surface.

2. Aecial primordia appear in the leaf as tangled wefts of hyphae on either side of the midrib. Nuclear migrations towards and into the primordium are common during its development.

3. The primordium becomes differentiated into an outer sterile region of plectenchyma and an inner pre-hymenial area. Cell fusions occur in the latter, and a hymenium of binucleate aeciosporophores is produced.

4. The aeciosporophores cut off first a peridial cap and then aeciospores, while the plectenchyma disintegrates. Intercalary cells are formed in the production of peridial cells and aeciospores.

5. Intercalary cells disintegrate to produce a protective gelatinous layer around the upper parts of the spore chains. This gelatin may also have some nutritive value.

6. A row of mature aecia with long cylindrical peridia breaks through the lower epidermis of the leaf on either side of the midrib.

7. Mature aeciospores are binucleate and possess thick, rough walls. During growth they develop three walls, the outermost disappearing at maturity.

8. Haustoria in the hemlock leaf are elongate and lobed uninucleate structures with an affinity for starch grains.

9. Aeciospores infect the hydrangea in early summer. Their germ tubes penetrate the leaf stomata and produce intercellular mycelia with binucleate cells and small reniform or pyriform, intracellular haustoria that are binucleate.

10. Uredinia develop from compact groups of swollen vertical hyphae which appear next to the lower epidermis. A compact peridium is formed, and uredospores with stalk cells are produced mainly by budding of the basal hymenial cells. Uredospores develop three walls, all of which persist in the mature spore.

11. Mature uredinia rupture the lower epidermis to expel spores which reinfect the hydrangea during the latter part of the summer. Their germ tubes penetrate the stomata.

12. In late summer and early fall, swollen vertical hyphae push up between palisade cells to the upper epidermis and give rise to binucleate intracellular teliospore initials. A single epidermal cell may receive several initials, and each initial generally becomes divided into a 2–8-celled teliospore.

13. Observations upon dividing nuclei in the teliospore initial reveal the presence of 4 chromosomes for the haploid number in this rust. The same number was found in *Thekopsora vacciniorum*.

14. When the teliospore initial ceases to divide, the wall of the teliospore begins to thicken, while the two nuclei fuse. During fusion, the chromatin of each nucleus condenses into a spireme-like structure and the nuclear membrane breaks down. A new nuclear membrane is formed in the fusion nucleus.

15. Teliospores overwinter in fallen hydrangea leaves to germinate the following spring and produce regular 4-celled basidia. Each basidium gives rise to 4 sporidia. The sporidia infect the hemlock.

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PLATE 11

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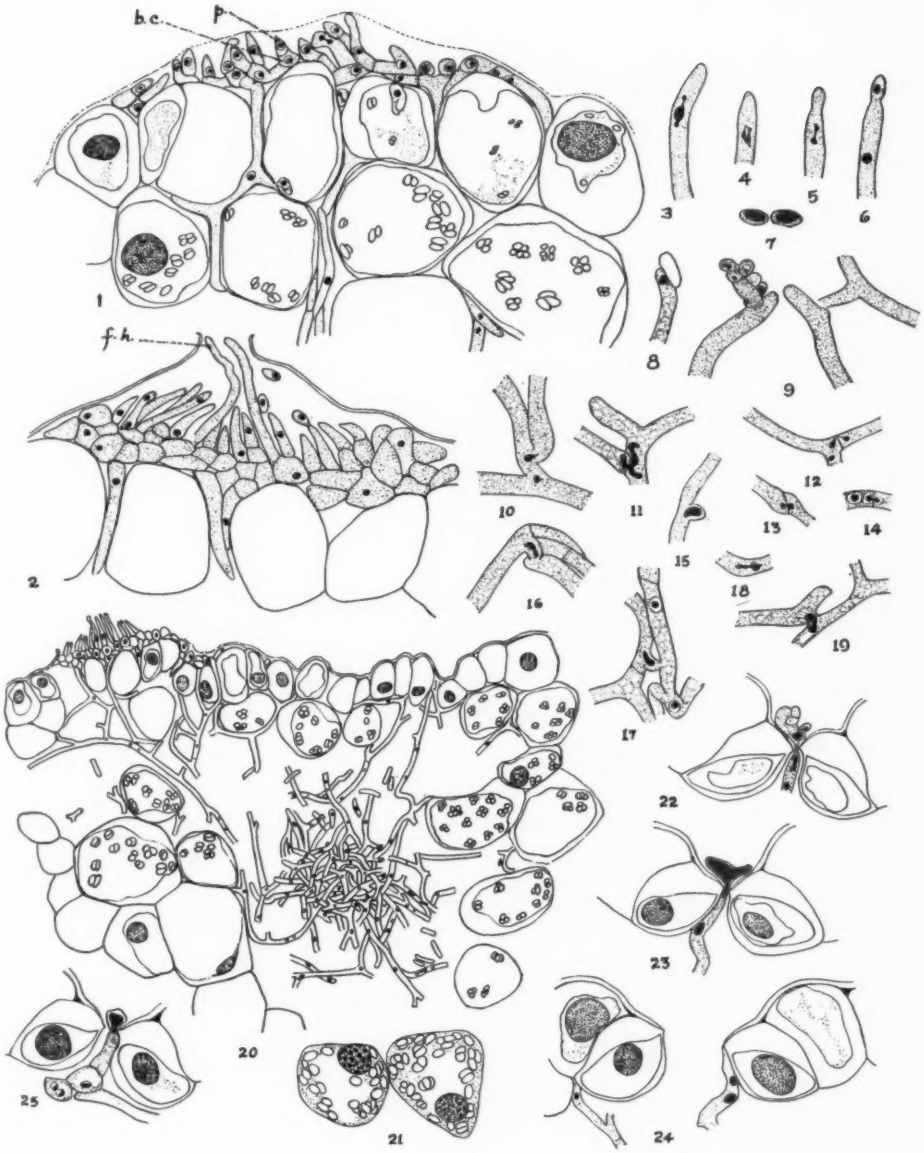
Figs. 112–113. Teliospore initial expanding within epidermal cell and receiving the two nuclei. × 1216.
Fig. 114. Two teliospore initials forming in the same epidermal cell. × 1216.
Fig. 115. Binucleate teliospore initial in epidermal cell; also a binucleate vertical hypha. × 1216.
Figs. 116–117. Anaphase nuclei in teliospore initials. × 1216.
Fig. 118. Early telophase, showing four expanded chromosomes at each pole. Note wall appearing across middle of spindle. × 1216.
Fig. 119. Telophase with polar radiations. × 1216.
Fig. 120. Late telophase following conjugate nuclear division in teliospore initial. Note cell wall appearing across middle of spindle fibers. × 1216.
Fig. 121. Teliospore initial, showing plasma membrane of host cell invaginated around it. × 1216.
Fig. 122. Binucleate teliospore cell with thickened walls. × 1216.
Figs. 123–127. Nuclear fusion in the teliospores. × 1216.
Fig. 128. Teliospore cell with a single fusion nucleus. × 1216.
Fig. 129. Section through a multicellular teliospore in an epidermal cell at about the time that nuclear fusion begins. × 540.
Fig. 130. Teliospore in lower epidermis. × 825.
Figs. 131–132. Abnormal development of teliospores in palisade region. (Dark inking indicates gelatinization of cells.) × 825.
Fig. 133. Abnormal development of teliospores in outer region of uredinium. × 825.
Fig. 134. *Thekopsora vacciniorum*. Teliospore initial, showing dividing nuclei and polar radiations. × 825.
Fig. 135. Same. Teliospore initial with dividing nucleus, showing the presence of four chromosomes at each pole and polar radiations. × 825.
Fig. 136. Same. Group of binucleate teliospore cells in lower epidermal cell of leaf of *Azalea*. × 540.
Fig. 137. Teliospore of *Thekopsora hydrangeae* producing a promycelium. Nucleus in prophase. × 1216.
Fig. 138. First meiotic division in the promycelium. × 1216.
Fig. 139. End of first meiotic division. Nuclei in brief resting stage. × 1216.
Figs. 140–141. Second meiotic division. One spindle is obliterated in the latter figure. × 1216.
Figs. 142–143. Four-celled basidia, producing sterigmata. × 1216.
Fig. 144. Live basidia producing sporidia. × 540.
Fig. 145. Sterigma with sporidium forming at the tip. Notice the attenuation of the nucleus as it passes into the sporidium. × 1216.
Figs. 146–147. Nuclear division in the sporidia just before the latter are discharged. × 1216.
Fig. 148. Binucleate sporidium with one of the two nuclei degenerating. × 1216.

PLATE 12

Fig. A. Section of a hemlock leaf, showing aecial primordium and pycnia.
Fig. B. Section through mature aecium, stained with safranin and gentian violet to show gelatinizing intercalary cells and extent of gelatin (dark band).
Fig. C. Mature pycnium in section of hemlock leaf.
Fig. D. Section of diseased hemlock leaf showing position of aecia and dark bands, indicating gelatinization, in the aecia.
Fig. E. Section of hemlock leaf, showing considerable hydrolysis of starch in the wing containing an aecium, and much less hydrolysis in less infected wing.
Fig. F. Section of hydrangea leaf, showing row of teliospores in upper epidermis.
Fig. G. Section of hydrangea leaf (lower surface up), showing mature uredinium.

PLATE 5



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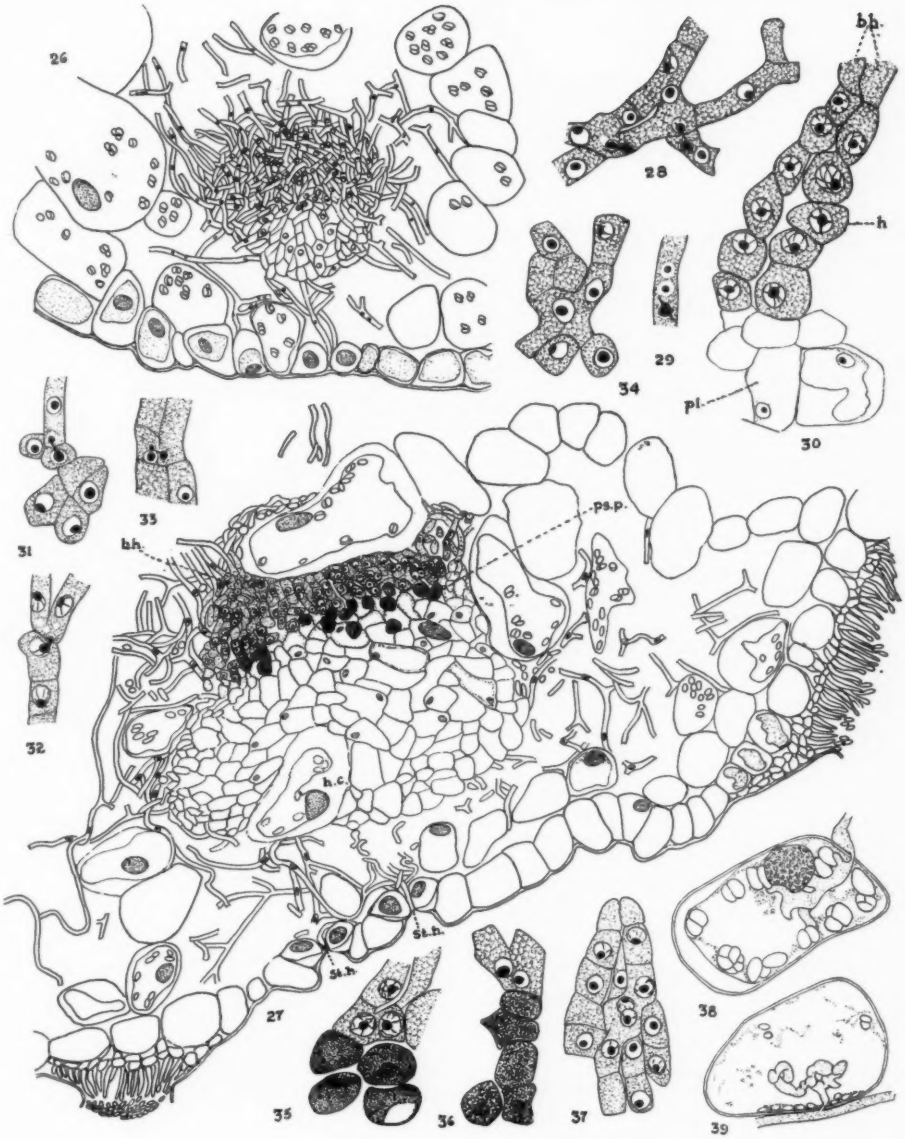

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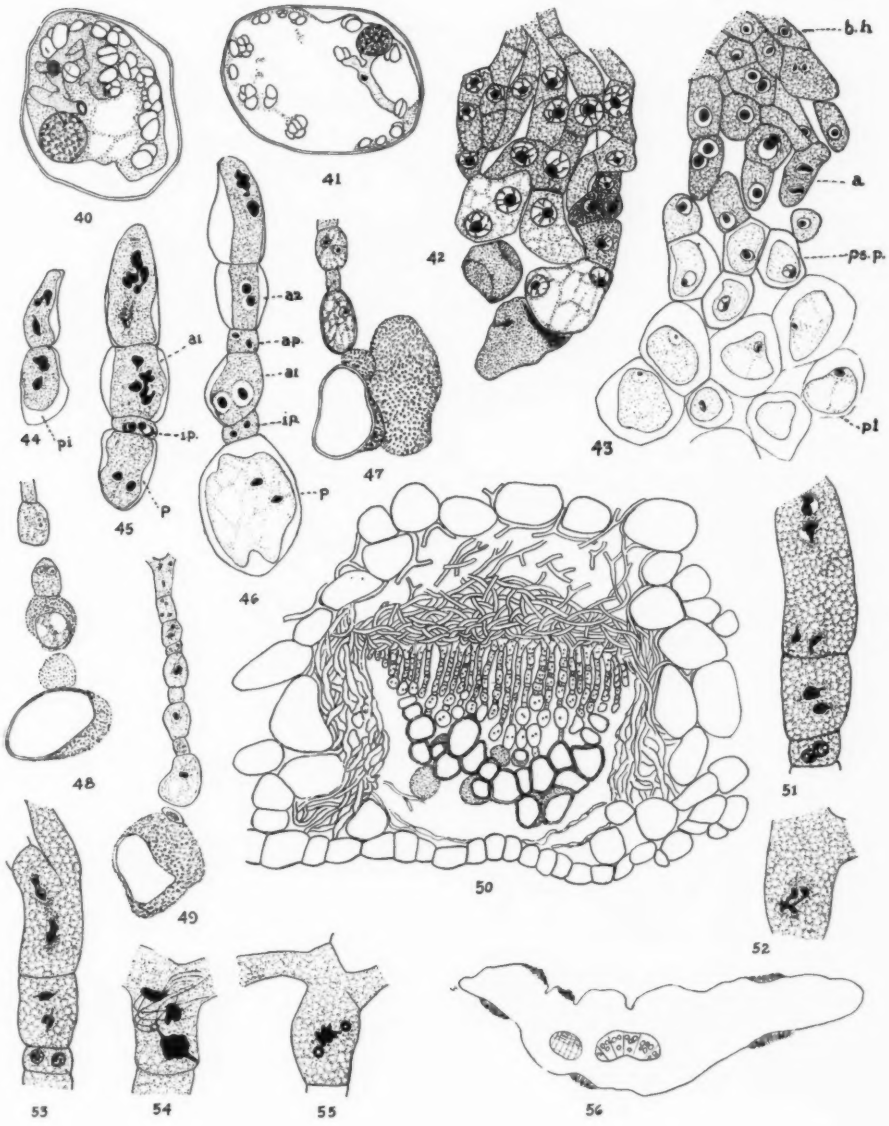
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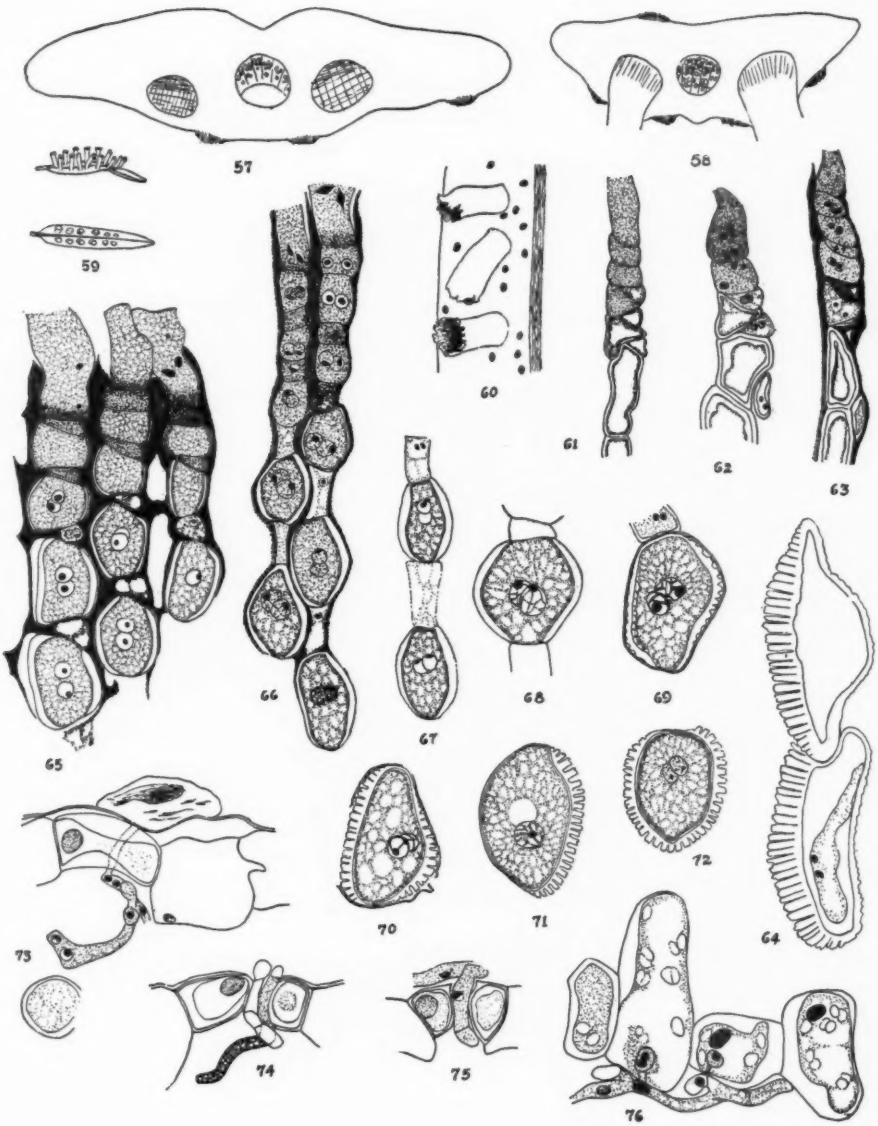
PLATE 8



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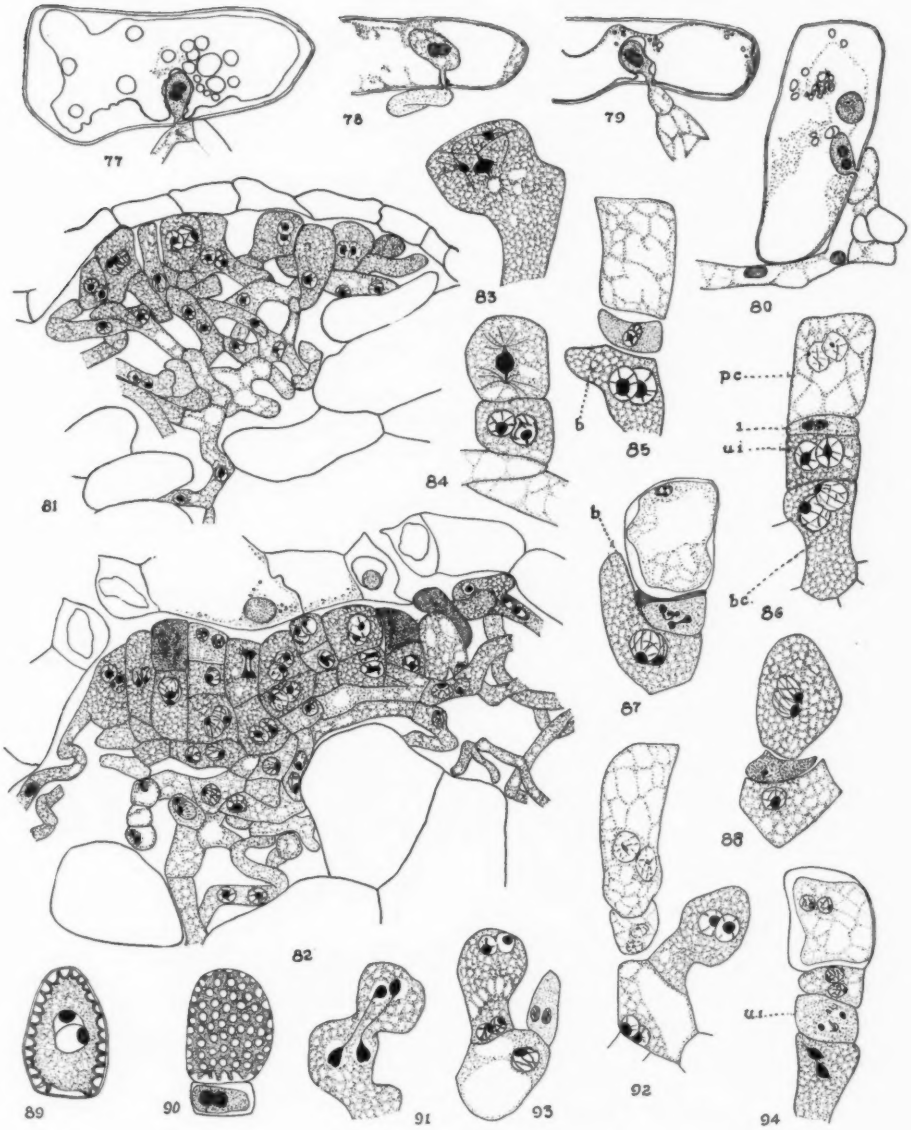

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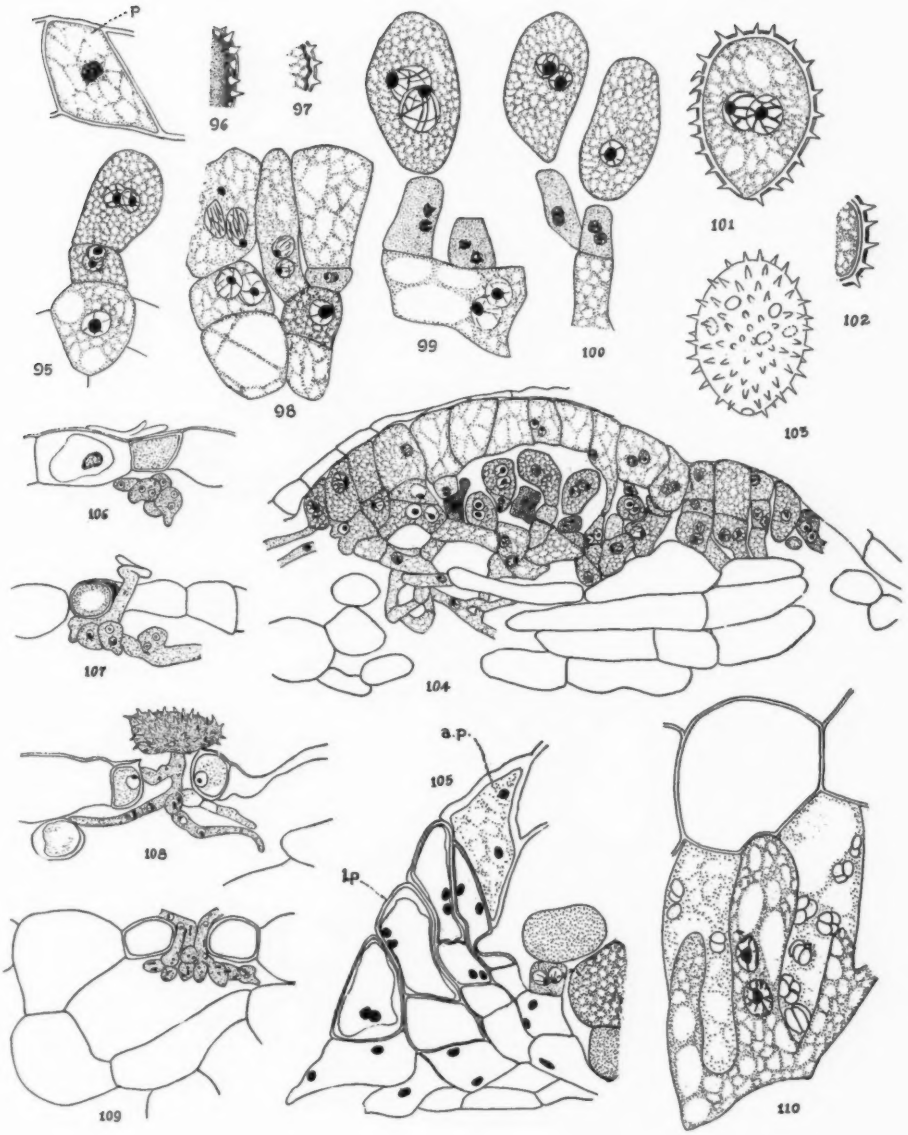

PLATE 11

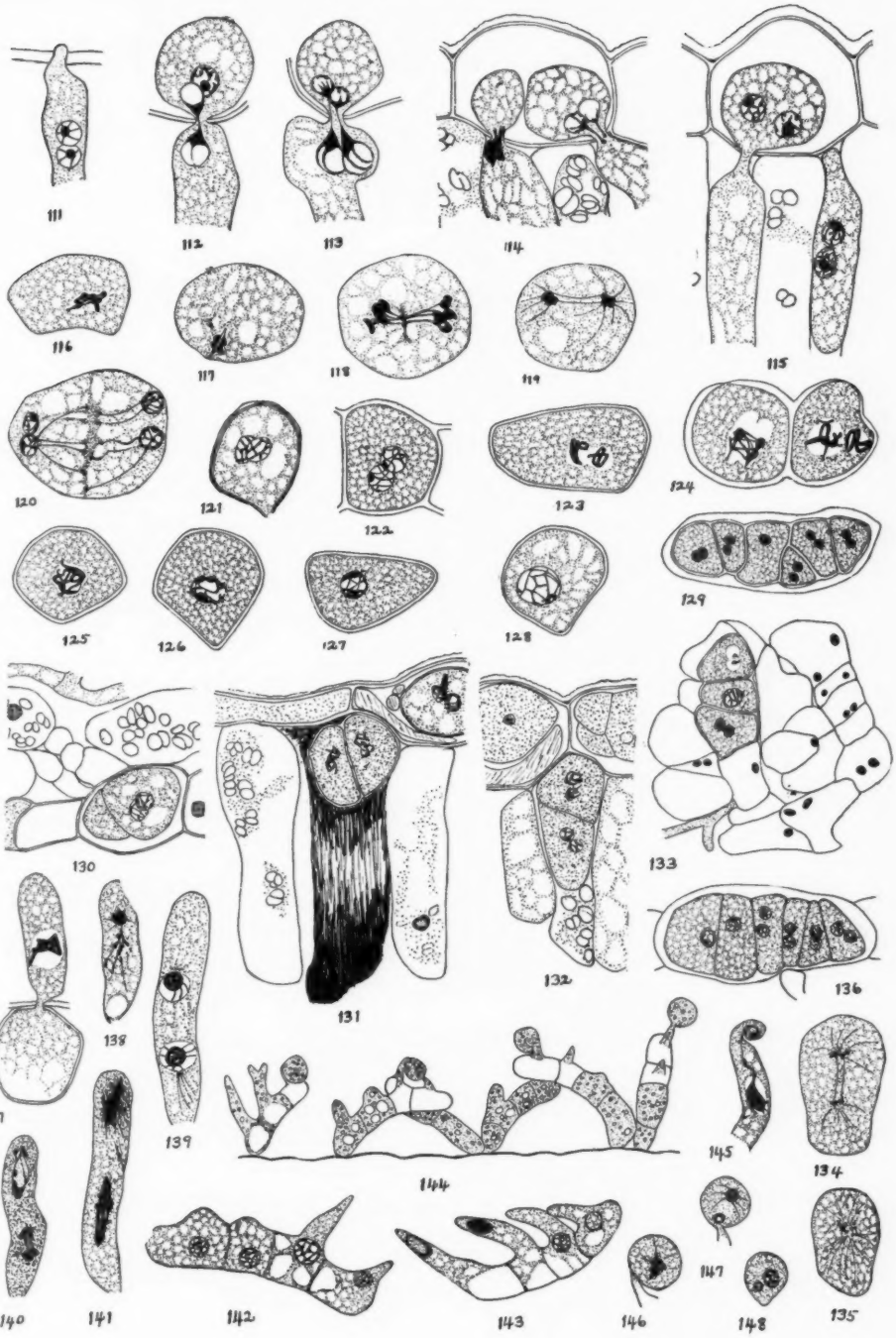
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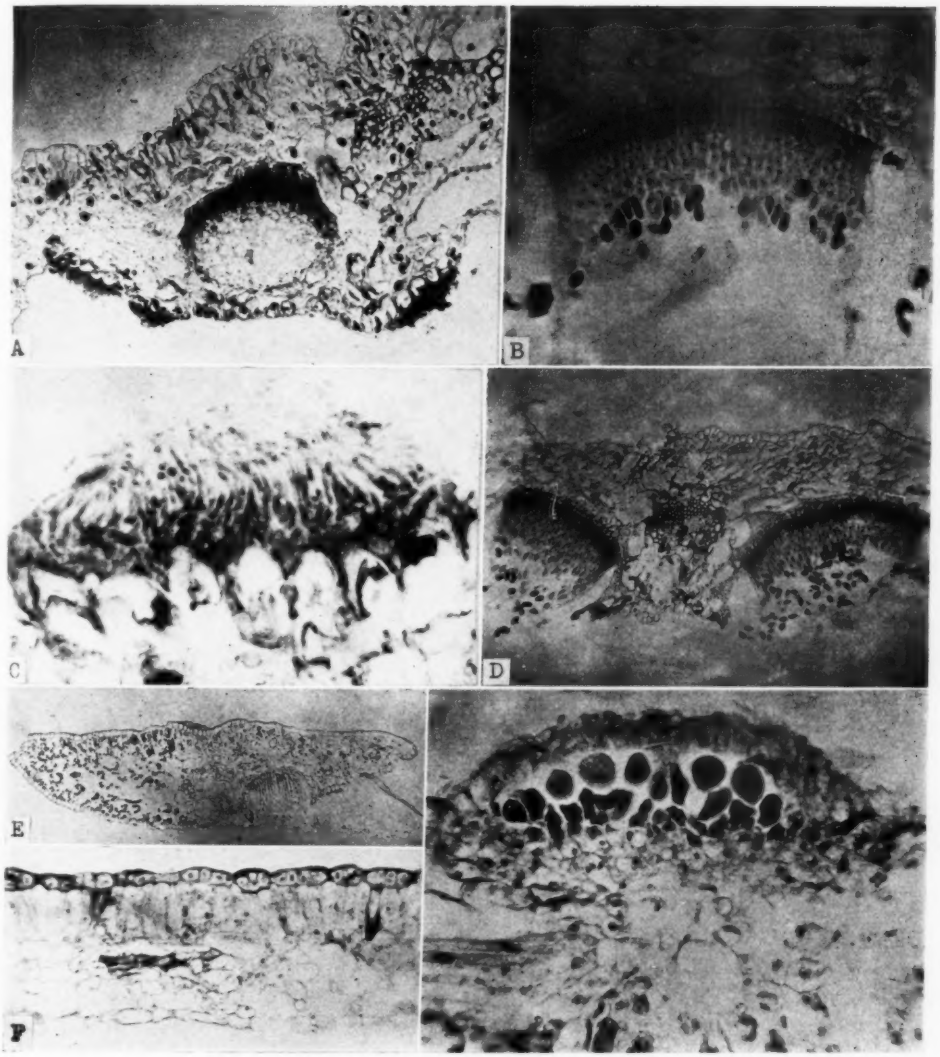
PLATE 12



AN F_2 COLCHICINE-INDUCED TETRAPLOID CABBAGE AND SOME COMPARISONS WITH ITS DIPLOID PROGENITOR

By Earl H. Newcomer

Plates 13–16

The importance of colchicine to practical as well as theoretical genetics is unquestioned. Despite the sometimes frantic claims made for it upon its recent introduction, it has effectively met a long felt need of cytologists for a method whereby polyploidy could be induced in many plants. At present it is being widely and successfully used and it is too early to attempt to evaluate its significance. Mention of several of the more important achievements secured through its use, indicating its practical and theoretical value to the cytologist and geneticist, should include such things as the rendering fertile of sterile hybrids through amphidiploidy, crossing hitherto uncrossable species through ploidy of one parent, production of triploids and other polyploids (3), the production of polysomics and the achievement of homozygosity in a single generation by treating haploids (4). Much of the literature has been recently reviewed by Dermen (2) and some of the physiological, morphological, and practical implications of the use of colchicine have been briefly reviewed by Nebel (3) and Barr and Newcomer (1, 5).

That increase in size which is usually but not always concomitant with polyploidy will, perhaps, not constitute the most important contribution of colchicine to the plant breeder, is suggested by recent studies on the physiological and nutritional aspects of polyploidy (1).

MATERIALS AND METHODS

Cabbage seedlings, variety Ferry's Hollander, were grown in flats in the greenhouse and while vigorously growing, were treated by pipetting the apical bud with a 0.2% solution of colchicine in the emulsion originated by Warmke and Blakeslee (6). This formula is superior to any others we have used and is as follows:

Stearic acid	3.00 grams
Morpholine	1.06 cc.
Tap water	40.00 cc.
Lanolin	16.00 grams

Add water to stearic acid and morpholine and heat mixture until the stearic acid is melted. Stir to a creamy soap solution. Add lanolin and continue heating until the lanolin is melted and the mixture is just below the boiling point. Stir to an emulsion and continue stirring intermittently until the emulsion is cooled to room temperature.

The emulsion must be diluted for spraying. Dilute by adding four parts of water to one of emulsion. The colchicine is then added to the desired concentration. This emulsion is very stable if kept in a cool place.

Applications of this mixture were made daily for three to six days. Upon the cessation of growth and the appearance of hypertrophy the plants were placed in the field where they matured with grossly abnormal, dichotomously branched stems and small, thickened leaves. In the fall, these plants were taken from the field, stored in a cold room until February when they were transferred to the greenhouse and forced for seed. In the cabbage, which is a biennial, the seeds thus obtained can be planted immediately and in this way two generations of progeny were grown in two years.

OBSERVATIONS

1. General morphological comparisons

	Diploid	Tetraploid
Flower petals	8 mm. x 22 mm.	10 mm. x 24 mm.
Pollen	24μ x 30μ	34μ x 40μ
Stomata	18μ x 21μ	26μ x 36μ
Plastids	ca 7μ	ca 7μ
Seeds	see fig. 21	see fig. 22

The flowers of the tetraploid, in addition to being conspicuously larger (cf. figs. 1 and 2), show other differences from the corresponding diploid. The sepals and petals are more divergent, the latter with obovate blades which taper toward the base, whereas in the diploid, the blades are nearly elliptical with crisped margins. The style of the tetraploid is distinctly curved at the tip, the anthers more strongly contorted and the corolla is more definitely salver-form.

Similarly, in all the other morphological comparisons made with the exception of the plastids, the tetraploid shows an advantage in size, most marked in the stomata (cf. figs. 3, 4), pollen grains (cf. figs. 5, 6 and 9, 10) and in general phenotypic appearance. There is no difficulty in distinguishing between the two in the field. The tetraploids are later in maturing (cf. figs. 17, 18), and the leaves are larger and thicker. This difference in thickness and stiffness of the leaves is apparent to the touch.

Seeds of the tetraploid average larger, although some of the larger diploid seeds equalled in size those of the smaller tetraploid seeds (cf. figs. 21, 22).

A cross section through the cotyledons (figs. 11, 12) of five-day-old seedlings shows that the increased thickness of the tetraploid cotyledons is due to an increase in cell size and not to an increase of number of cells. In numbers of cells they seem to be approximately equal.

Observations on the comparative behavior of the chromosomes during meiosis were only preliminary to a more detailed study to be made later. There were evidences of multivalent associations (fig. 13) with quadrivalents the most common. Disjunction appeared to be normal and functional gametes formed. Fertility of greenhouse-grown plants was high, but seldom exceeded 70% of complete fertility.

Crosses between the tetraploid and diploid gave seed only when 2n pollen was used, but to date none of the 3n seeds have produced viable seedlings.

2. Physiological comparisons

As to the internal, physiological consequences of the new genetic regime established by polyploidy, we have only a meager and superficial knowledge from which no generalizations are possible at the present time. Recent comparative studies of these cabbages (1), in such things as reducing sugars, acid hydrolyzable substances, soluble nitrogen, residual nitrogen and vitamin C revealed that the tetraploid cabbage contained higher concentrations of all substances, save soluble nitrogen, when tested on a fresh weight basis. Vitamin C content of the lower green leaves of the tetraploid was found to be slightly over four times that of the corresponding diploids. Increases in the other analyzed substances, save soluble nitrogen, averaged between 25–50% in the autotetraploid. The possibilities of this aspect of polyploidy in a plant breeding program from the point of view of general nutrition, drug production, etc., seem to be as great as they are obvious.

SUMMARY

The colchine-induced autotetraploid cabbage of this study was larger in every morphological comparison made, with the exception of chloroplasts, than the corresponding diploid progenitor. In addition to quantitative morphological changes, flowers show certain qualitative distinctions. Difference in thickness of the cotyledons and also presumably the leaves, was not due to increase in cell number but to increase in cell size. With the exception of primary multivalent pairing, chromosome behavior was normal, producing functional gametes and about 70% of complete fertility.

Physiological studies, in which the 4n and 2n cabbages were analyzed for reducing sugars, total sugars, acid hydrolyzable substances, soluble nitrogen, residual nitrogen and vitamin C revealed that, with the exception of soluble nitrogen, the tetraploid contained from 25% to 50% more of these substances. The lower leaves of the tetraploid contained four times as much vitamin C as the corresponding leaves of the diploid.

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EXPLANATION OF PLATES

All figures are paired. The odd numbered figures on left side of plates are tetraploid; those on the right side and even numbered are diploid.

PLATE 13

Fig. 1. Flower of tetraploid plant.
Fig. 2. Flower of diploid plant.
Fig. 3. Stomata, guard cells and lower epidermal cells of tetraploid, × 675.
Fig. 4. Stomata, guard cells and lower epidermal cells of diploid, × 675.
Fig. 5. Pollen grains of tetraploid, × 150.
Fig. 6. Pollen grains of diploid, × 150.

PLATE 14

Fig. 7. Chloroplasts of tetraploid, × 675.
Fig. 8. Chloroplasts of diploid, × 675.
Fig. 9. Pollen grains of tetraploid, × 675.
Fig. 10. Pollen grains of diploid, × 675.

PLATE 15

Fig. 11. Cross section through 5-day-old cotyledon of tetraploid, × 150.
Fig. 12. Cross section through 5-day-old cotyledon of diploid, × 150.
Fig. 13. Chromosomes in first meiotic metaphase of tetraploid, × 675.
Fig. 14. Chromosomes in late diakinesis of diploid, × 675.
Fig. 15. Chromosomes in second metaphase of tetraploid, × 675.
Fig. 16. Chromosomes in second metaphase of diploid, × 675.

PLATE 16

Fig. 17. Field grown tetraploid cabbage.
Fig. 18. Field grown diploid cabbage of same age as plant in fig. 17.
Figs. 19, 20. Two rows of tetraploid and diploid plants in the field, respectively.
Fig. 21. Seeds of tetraploid.
Fig. 22. Seeds of diploid.

PLATE 13

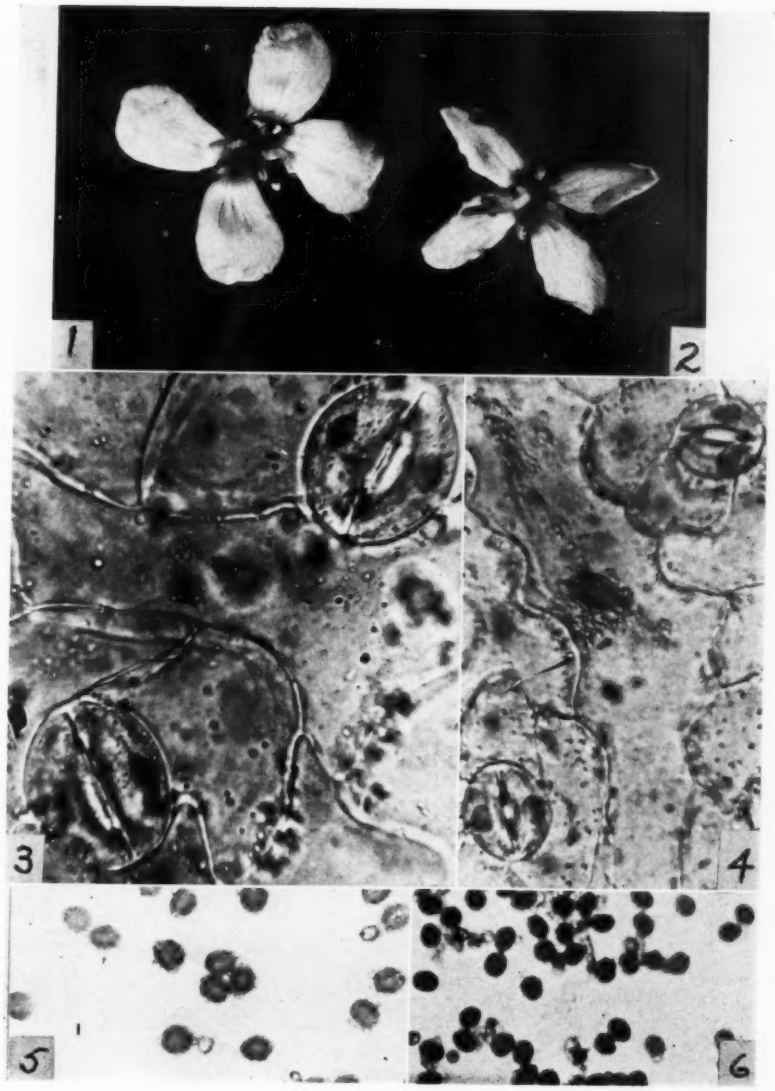

PLATE 14

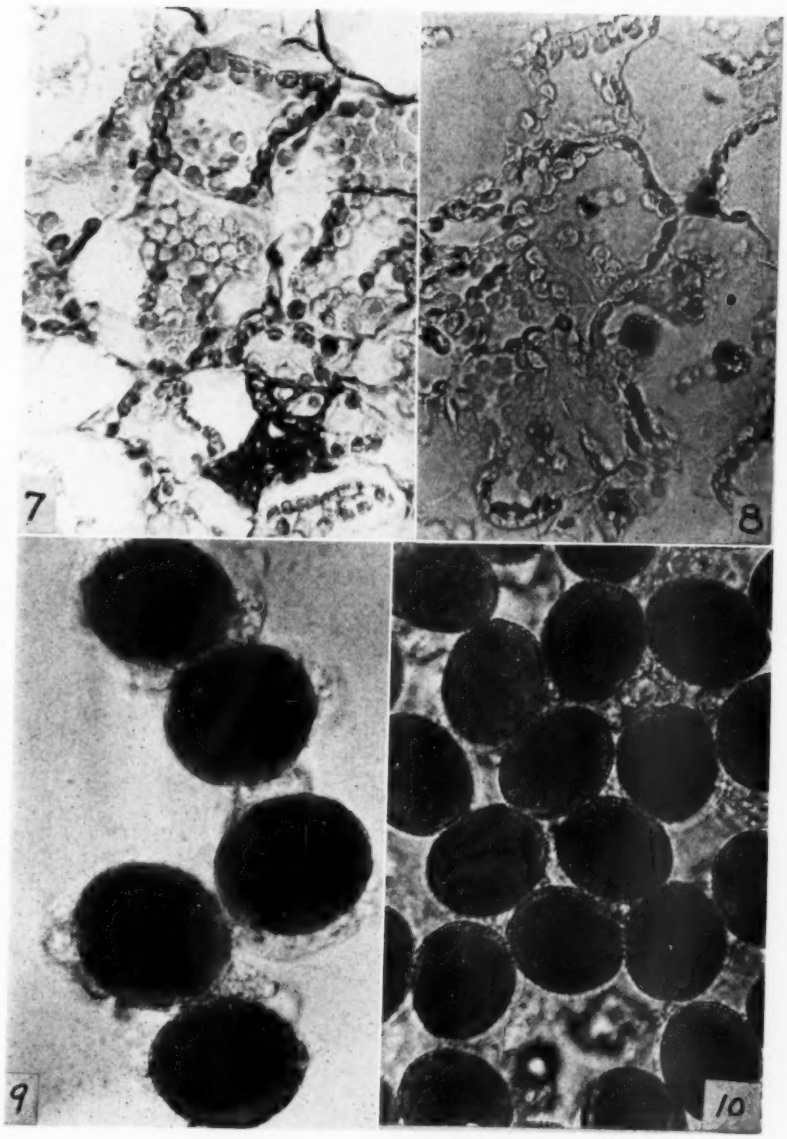

PLATE 15

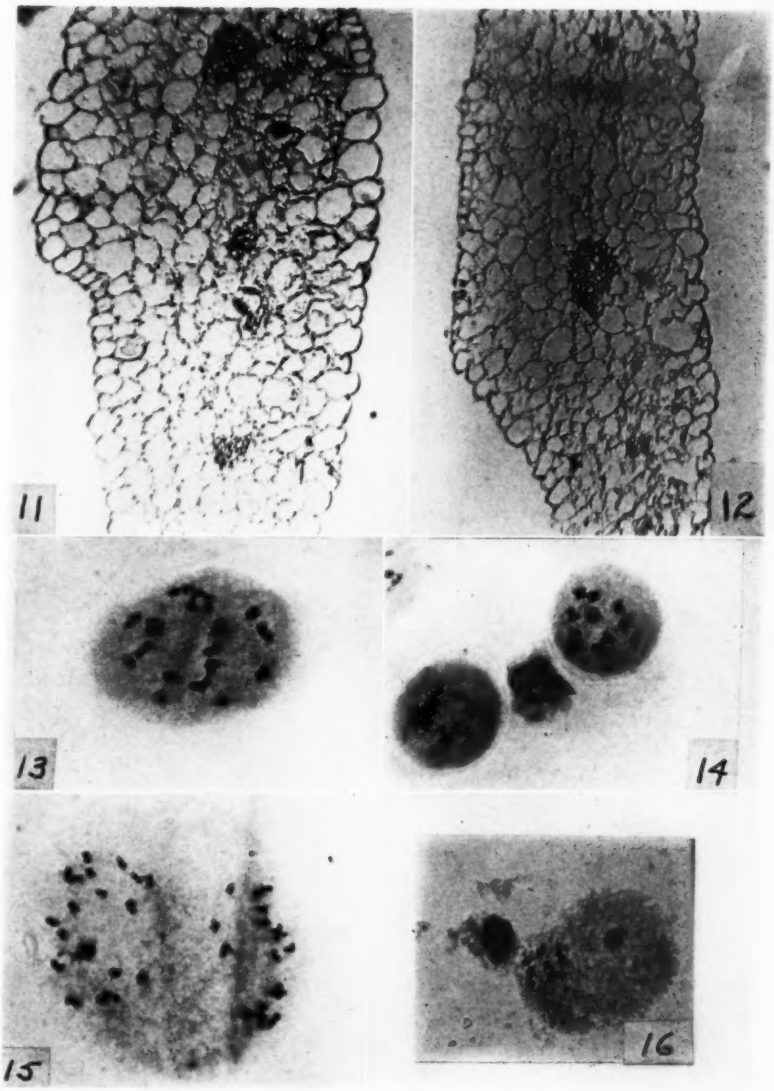

PLATE 16

STUDIES OF NATURAL POPULATIONS OF CUTHBERTIA GRAMINEA IN THE CAROLINAS

By Norman H. Giles, Jr.

Two Text Figures

Cytotaxonomic and cytogenetic studies of natural plant populations are essential in any attempt to evaluate the relative importance of genetic mechanisms responsible for variation and for evolution, especially at and below the species level. One of the most widespread of these mechanisms in the flowering plants is polyploidy—both the auto—and allotypes. Consequently, a proper evaluation of its role in speciation is of considerable importance (cf. Stebbins, 1940). A study of polyploidy in natural populations is of further interest, since such an investigation often makes possible, when correlated with geographical and geological data, a rather precise interpretation of the evolutionary history of a given species or group of related species (Manton, 1934; Stebbins, 1942).

The present discussion is concerned with autopolyploidy within the taxonomic species, *Cuthbertia graminea* Small. Furthermore, it is restricted primarily to a consideration of the extent and significance of the geographical ranges of the various cytological forms of the species found in the Carolinas. The more general conclusions have already been presented (Giles, 1942). It has been shown as a result of extensive collections of living material for cytological examination that *C. graminea* occurs in nature in two principal forms, a diploid and an autotetraploid. A comparison of these two forms shows that the tetraploids differ from the diploids in several respects: (a) they are larger, more vigorous plants; (b) they are more variable cytologically; (c) they exhibit a greater ecological amplitude; (d) their geographical range is much greater, and further, the ranges of the two forms (in so far as the collections up to that time indicated) are distinct. More collections have been made since the paper cited was published, and it is now clear that the two ranges are indeed strikingly distinct, and that the diploids are confined to the Carolinas. Furthermore, the range of the diploids has been delimited with considerable exactness.

DATA ON COLLECTIONS

With the assistance of several cooperative collectors it has been possible to secure living specimens of *C. graminea* from many different stations in the Southeast. An extensive collection was possible largely because of the relative ease with which the plants can be obtained during trips by automobile. The species is almost entirely confined to a characteristic vegetation-habitat complex: the wiregrass, turkey-oak, long-leaf pine association of xeric sandy areas on the Coastal Plain (cf. Wells and Shunk, 1931). These areas can be easily recognized as one travels through the region, and stops can be made at frequent intervals to collect living plants for subsequent examination in the laboratory. Many of the collections were grown at the Arnold Arboretum of Harvard University and at the Marsh Botanical Gardens of Yale University.

TABLE 1

Data on collections of Cuthbertia graminea in the Carolinas

Under locality, the distance and direction of the collecting station from a nearby point of reference are given; numbers refer to federal (U.S.) and state highways. Abbreviations of collectors' names are as follows: R = Mr. H. A. Rankin; K = Dr. Thomas Kerr; S = Dr. C. P. Swanson; O = Dr. H. J. Oosting; T = Mr. F. G. Tarbox, Jr.; B = Mr. R. K. Godfrey; G = the writer and his wife. For further explanation see text.

COLLECTION NAME	NO. OF PLANTS	CHROM. NO.	LOCALITY	COLLECTOR
Rankin	3	2x	Lillington, N. C., 7 mi. *S* U. S. 15A	R
Harnett	3	2x	Spout Springs, N. C., 2 mi. *SE* State 87	K, S, G
Fort Bragg	7	2x	Fort Bragg, N. C., State 87	K, G
Moore	1	2x	Moore County Line, N. C., *S* State 211	K, G
Aberdeen	2	2x	Aberdeen, N. C., 3 mi. *S* U. S. 15	K, G
Sanatorium	2	2x	Sanatorium, N. C., 2 mi. *SE* State 211	K, G
Raeford	2	2x	Raeford, N. C., 2 mi. *NE* U. S. 15A	K, G
Hoke	3	2x[a]	Hoke County Line, N. C., *N* U. S. 15A	K, G
Hoffman	1	2x	Hoffman, N. C., 3 mi. *NE* U. S. 1	K
Marston	1	2x	Marston, N. C., 3 mi. *SW* U. S. 1	K
Scotland	1	2x	Laurinburg, N. C., 10 mi. *N* U. S. 15A	K, G
Robeson	2	2x	Rennert, N. C., pocosin 1 mi. *E*	K, G
Hamlet	4	2x	Hamlet, N. C., 2 mi. *E* U. S. 74	G
Old Hundred	3	2x	Old Hundred, N. C., U. S. 74	G
Cash	4	2x	Cash, S. C., U. S. 52	G
Bear Creek	6	2x	Chesterfield, S. C., 8 mi. *S* State 102	G
Sandhills	3	2x	Patrick, S. C., 4 mi. *SE* State 102	G
Coker	1	2x	Hartsville, S. C., ½ mi. *N* U. S. 15	G
McBee	6	2x	McBee, S. C., 5 mi. *SE* State 151	G
Pearce	3	4x	Fayetteville, N. C. (near)	R
Vander	1	4x	Vander, N. C., State 24	K, G
Cumberland	4	4x	Fayetteville, N. C., 5 mi. *E* State 24	K, S, G
Fayetteville	3	4x	Fayetteville, N. C., 5 mi. *S*	R
Rex	3	4x	Rex, N. C., pocosin rim near	K
St. Paul's	2	4x	St. Paul's, N. C., 4 mi. *E* State 20	K
Page's Lake	3	4x	Bladen County, N. C., *NW* corner, State 20	K
Kenan	8	4x	Kenansville, N. C., 8 mi. *S* State 11	K, S
Burgaw	5	4x	Burgaw, N. C., by North East Cape Fear River, State 53	K, S
White Lake	3	4x	White Lake, N. C.	R
Bladen	1	4x	Black River at Beatty's Bridge, N. C.	R
Warwich Bay	2	4x	Lumberton, N. C., 10 mi. *SE*	K
New Hanover	3	4x	Carolina Beach, N. C., 3 mi. *N*	O
Carolina Beach	2	4x	Carolina Beach, N. C. ½ mi. *N*	K, S
Brunswick	1	4x	Southport, N. C., 4 mi. *W*	K, G
Laurinburg	1	4x	Laurinburg, N. C., 5 mi. *SW* U. S. 15	G
Society Hill	1	4x	Society Hill, S. C., 1 mi. *SW* U. S. 15	G
Segars	7	4x	Segars, S. C., 2 mi. *W* State 151	G
Chesterfield	2	4x	McBee, S. C., 1½ mi. *SW* U. S. 1	G
Lynches	4	4x	McBee, S. C., 3 mi. *SW* U. S. 1	G
Bethune	12	4x	Bethune, S. C., 2 mi. *SW* U. S. 1	G
Cassatt	2	4x	Cassatt, S. C., 5½ mi. *SW* U. S. 1	G

TABLE 1—*Concluded*

COLLECTION NAME	NO. OF PLANTS	CHROM. NO.	LOCALITY	COLLECTOR
Kershaw	2	4x	Camden, S. C., 5 mi. *NE* U. S. 1	G
Camden	2	4x	Camden, S. C., 4 mi. *NE* U. S. 1	G
Blaney	3	4x	Blaney, S. C., 3 mi. *NE* U. S. 1	G
Columbia	1	4x	Columbia, S. C., 2 mi. *NE* U. S. 1	G
Congaree	6	4x	Columbia, S. C., 5 mi. *W* U. S. 1	G
Lexington	4	4x	Lexington, S. C., 5 mi. *W* U. S. 1	G
U. S. 1	3	4x	Monetta, S. C., 2 mi. *S* U. S. 1	G
Monetta	3	4x	Monetta, S. C., 3 mi. *S* U. S. 1	G
Edmund	1	4x	Edmund, S. C., 3 mi. *N* State 215	G
Aiken	9	4x[b]	Aiken, S. C., 5 mi. *NE* U. S. 1	G
South Fork	2	4x	Aiken, S. C., 8 mi. *E* State 215	G
Tarbox	1	4x[c]	Brookgreen, S. C.	T
Godfrey	1	4x	Georgetown, S. C., 10 mi. *N* U. S. 17	B
Georgetown	3	4x	Georgetown, S. C.	B

[a] 1 = 3x.
[b] 1 = 6x; 1 = 4x + 1.
[c] Data from Anderson and Sax (1936).

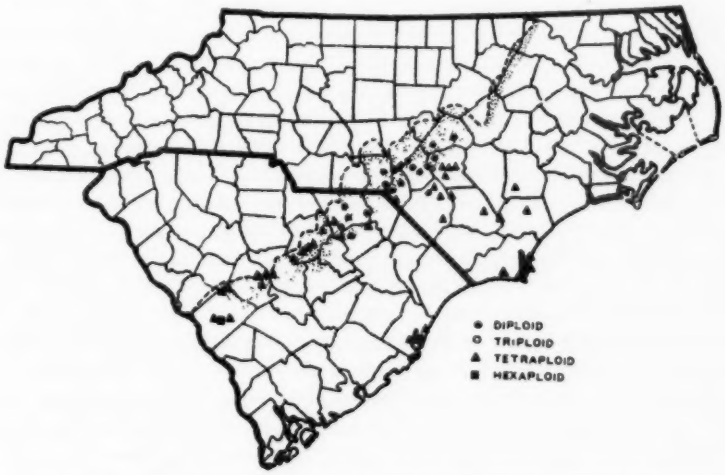

FIGURE 1. Distribution of the cytological forms of *Cuthbertia graminea* in the Carolinas. The approximate position of the Fall Line is shown by the broken line, and the approximate limits of the surficial Cretaceous sediments by stippling.

The data on all collections which have been made in the Carolinas are presented in table 1. A collection name has been assigned to all the plants from one station. The number of plants at each station for which successful cytological determinations were obtained is then given, followed by the chromosome numbers of the plants. Next the location of the collection is given in some detail,

special reference being made to state and national highways along which most of the collections were made. The last column indicates by letter the name of the collector. The collections are arranged with diploid stations first, in approximate geographical order from north to south, followed by the tetraploid stations arranged in the same way. In all there are 55 stations in the Carolinas, 19 being diploid localities and 36 tetraploid localities. A total of 169 plants has been examined cytologically. The locations of the stations are indicated on a county outline map of the Carolinas in figure 1.

DISCUSSION

It is at once clear from the collection data and from the ranges indicated on the map that the two cytological forms occupy distinct areas. In no instance have they yet been found growing together at the same station, although several of the stations of the two types are only a few miles apart. There is, however, some evidence that crossing of the two forms occurs naturally. A single triploid individual was found at the Hoke station, which is on the periphery of the diploid range where it comes in contact with the range of the tetraploids. This one example is not sufficient to indicate that extensive hybridization of the two types occurs, since relatively few plants from the zone of contact of the two populations have been examined cytologically. Even if crossing does occur in certain areas with considerable frequency, it is of course clear that the two types will remain effectively isolated genetically, since the high degree of sterility of the hybrid triploids (which have been produced and tested experimentally) would almost entirely preclude any exchange of genes between the two populations.

It is clear that a form such as an autotriploid with an odd number of chromosome sets could not become an established element in populations of a sexually reproducing species. However, higher autopolyploids with an even number of chromosome sets do possess the possibility of fairly regular sexual reproduction. Autohexaploids could arise by somatic chromosome doubling in triploids resulting from hybridization of diploids and autotetraploids. Such hexaploids would then be expected to occur where the ranges of the two other forms meet. An example of this sort is found in *Sedum pulchellum* Michx. (Baldwin, 1943). Hexaploids might also arise occasionally in a pure tetraploid population as a result of the union of a tetraploid and a diploid gamete. Such individuals would be rare, and the chances of hexaploids becoming established in a population in this manner would appear to be very slight. The single hexaploid found in this study (at the Aiken station) clearly did not arise from hybridization, since the locality where it was found is considerably beyond the limits of the diploid range. It must be a sporadic case of the sort just mentioned. There is no present evidence that *autohexaploids* constitute a real element in the natural populations of *C. graminea*. If they existed in any number it would appear that they should have been found in the zone where the diploid and tetraploid forms meet, and such has not been the case. It should be pointed out that the hexaploid individual reported previously from central Florida has been found to be part of a hexaploid population and originated in an entirely different manner genetically from the Aiken autohexaploid (Giles, unpublished).

Certain important characteristics of the range of the diploids may now be pointed out. It is evident that this form occupies a much smaller area than do the tetraploids, especially when it is recalled that the range of the latter extends well into Florida. Further, the diploids are almost entirely confined to the Sandhills adjacent to the Fall Line, from the vicinity of Lillington, N. C., to McBee, S. C., whereas the tetraploids occur on the Coastal Plain terraces as well. As was pointed out previously (Giles, op. cit), there is a clear correlation between the geology of the region and the geographical and cytogenetical data. The diploids, which are known from genetic evidence to be the ancestral type, occupy an area which is much older geologically than that in which the majority of the tetraploids occur. The Sandhill area of the Carolinas, the northern part of which was formerly included in the Lafayette formation (Stephenson, 1912) is now considered to be largely Upper Cretaceous and subordinately Paleocene in age (Cooke, 1936; Stephenson, 1940). It is probable that this region has not been under the sea since early Tertiary at the latest. The region between the surficial Cretaceous sediments and the present coast line is much younger in age; most of the coastal terraces, which occupy a considerable fraction of this area, being of Pleistocene origin. The diploids must represent then the ancestral population and its descendants, which now occupy entirely or in part their original range in a region geologically old, whereas the derived and more vigorous autotetraploids have been the effective colonizers of extensive new areas as they became available for occupation by land plants.

In order to show quite clearly the extent of the correlation between the range of the diploids and the geology of the Carolinas, figure 2 is presented. On this map the approximate limits of the surficial Cretaceous sediments are indicated by stippling, and the extent of the diploid area is shown by oblique lines. It can be seen that the area which the diploids occupy in southern North Carolina and in northern South Carolina as far south as McBee parallels quite closely (with the exception of the Robeson station) the limits of the Cretaceous-Paleocene sediments, and it was at first thought that this form would occur throughout the Cretaceous area. South of McBee, however, tetraploids are found in the Fall Line Sandhills. As far as is known at present there are no fundamental geological differences which distinguish these two Sandhill regions. About the only differences seem to be topographic and edaphic ones, since the topography and soil zones are more continuous in the diploid area, less cut to pieces by transverse streams, and wider, in general, than in the area to the northeast or southwest (Eargle, 1942). One major question then remains: Did the diploids once occupy all the Fall Line Sandhill region and perhaps a considerable portion of the adjacent regions as well (having been displaced sometimes in the past from a part of their range by the tetraploids), or does their present range represent the entire extent to which they spread in the past, the tetraploids having originated somewhere along the present zone of contact and then having colonized the remainder of the Coastal Plain? It does not seem possible to decide between these two alternatives as yet. However, a method of attacking the problem does suggest itself. Since the present limits of the diploid area are now known with considerable exactness, it would seem that further population studies in the

region where the two types approach one another might provide valuable information as to the present relation between them. More information is desirable concerning conditions on the periphery of the diploid range, especially at two points: the outlying Robeson station and adjacent Hoke and Scotland counties in North Carolina; and the McBee-Hartsville region (Segars, Chesterfield, Coker, and McBee stations) at the southern limit of the diploid range in South Carolina. Is there any evidence that the tetraploids are replacing the diploids in localized areas? How do the reproductive potentials of the two types compare? Ecological studies, perhaps over a period of years, might indicate actual shifts in the relations of the populations. Such information, besides making possible a more exact interpretation of the history of the diploid form, should be of quite

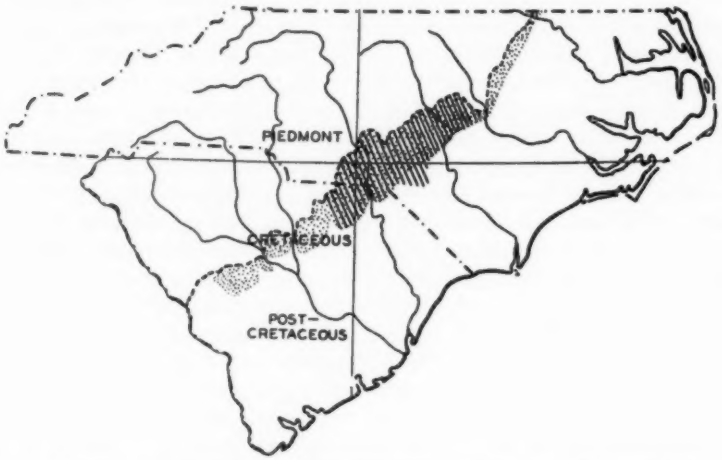

FIGURE 2. The diploid area of *Cuthbertia graminea*. The extent of this area is shown by oblique lines. Other symbols as in figure 1.

general interest, since it bears directly on the larger problems of how competition and selection operate in nature.

One more aspect of the present study should be mentioned. There is now botanical as well as geological evidence that the Fall Line Sandhill region is older than the rest of the Coastal Plain. If there is one relic plant population present in this region, may there not be others? Indeed, the recent extensive study of natural populations in the genus *Vaccinium* by Camp (1942) indicates that in this genus at least one of the diploid ancestral species found in the Southeast may be centered in the Sandhill region. The discovery of further examples of polyploid complexes and of relic populations may be anticipated in the southeastern Coastal Plain flora. It may well be that the combined evidence from such studies will facilitate an interpretation of the history of many of the species and perhaps of the general flora in this region with a much greater degree of certainty than has hitherto been possible.

SUMMARY

Studies of natural populations of *Cuthbertia graminea* have demonstrated the existence of autopolyploidy within this taxonomic species, diploid, triploid, tetraploid, and hexaploid forms having been found. Extensive collections of living plants from throughout the southeastern Coastal Plain have shown that the diploids and tetraploids are the only abundant forms. Furthermore, it is now clear that the two forms occupy distinct regions. The diploids are confined to a relatively small area in the Fall Line Sandhills of the Carolinas, whereas the tetraploids occur in the remainder of the Coastal Plain, well into Florida. There is some evidence that occasional hybridization of the two types may occur in the zone where the two populations meet, a single triploid having been found at the Hoke station in North Carolina on the periphery of the diploid range. There is no evidence as yet that autohexaploids constitute a significant element in the species population, the single individual found at the Aiken station in South Carolina apparently representing an exceptional case which arose spontaneously in a tetraploid population.

A correlation of evidence from cytogenetics, geographical distribution, and geology indicates that the diploid form is largely confined to a small area which is geologically old (Cretaceous), whereas the derived tetraploid occupies a much larger area which is for the most part much younger in age (Pleistocene). The advent of autotetraploidy has been of major importance in the history of the *C. graminea* complex, since the demonstrably more vigorous tetraploids have been able to effect a great extension of the species range by colonizing new areas as they became available for occupation by land plants. Consequently, the tetraploids are now the dominant form in natural populations of this species, both numerically and in geographical range.

The relation between the two forms under the influence of natural selection is not yet clear, but there is some evidence from distribution that the tetraploid may have displaced the diploid from some of the latter's original range. The suggestion is made that further population studies in the zone where the two types meet might indicate whether such a displacement is taking place at present. Finally, it is pointed out that the Fall Line Sandhills in the Carolinas may well contain relic populations of other plant species, especially those in which polyploid complexes exist.

Acknowledgments. The writer would like to express his appreciation to the individuals listed in table 1 who have been of invaluable assistance in collecting living specimens. He is also indebted to the staff of the Department of Biology, Emory University, for facilities and materials used in the cytological examination of many of the collections.

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MAGNOLIA CORDATA MICHAUX

By W. C. Coker

Plates 17–20 and 6 Text Figures

Even as late as 1928 R. M. Harper spoke of *Magnolia cordata* as "this little-understood and somewhat mysterious species, or perhaps only a variety." Since that time a good deal has been found out about *M. cordata*, and the known distribution of the typical tree, or very slight variations from this tree, has been extended both eastward and westward from a small group of counties near Augusta, Georgia. It is now known to occur in the vicinity of Athens and several counties below this area, and more recently a very interesting extension has been discovered in central South Carolina. We are publishing herewith a map giving the present known range and including locations for all actual specimens we have been able to examine and also what we consider authentic records, though not yet supported by specimens we have seen. Before going into any detail as to these new discoveries and any additional knowledge brought out by them, we might first review the history of *Magnolia cordata*.

It was first named in the elder Michaux's Flora of North America in 1803. The very brief description in Latin (translation: leaves cordate, subtomentose beneath; flowers yellow) might as easily refer to what we are now calling the yellow-flowered variety of *M. acuminata* (*Tulipastrum acuminatum* var. *aureum* Ashe, 1917; later as *M. acuminata* var. *aurea* in 1931). Sargent (1891) says that the specimen in Michaux's herbarium in Paris is *M. acuminata*.

The younger Michaux in his North American Sylva (1857, first English edition, translated from first French edition of 1810) described *M. cordata* more fully and included an illustration in color. In this plate the flower is rather poorly done, in both form and color.

In neither of these works is it possible to tell whether *M. cordata* or *M. acuminata aurea* or both are involved. Fortunately however F. A. Michaux mentioned a particular station, "the plantation of *Good-rest*, twelve miles from Augusta, where, in my last journey to the United States, I noticed it along the sides of *Horn Creek*." This station is in Edgefield County, S. C., only about 20 miles from Spear's plantation where Berckmans rediscovered *cordata* on the other side of the river. It is only about 10 miles from where Ashe found it again in 1931 on the South Carolina side of the river near Augusta. It would seem permissible then for us to consider the tree found in this area typical, so far as we will ever know, of *M. cordata*. Up until the rediscovery by Mr. Berckmans, the tree was entirely unknown in the wild state. However, it had been introduced into cultivation in Europe by Michaux or by the Scotch collector John Fraser or probably by both (Loudon says it was introduced into England by John Lyon), and had later been brought back to America. Sargent (1889) says that when Asa Gray took charge of the Harvard Botanic Garden in 1842 he found there two already well grown trees of *M. cordata*. It was from one of these trees that

Sargent's description and Faxon's fine figure were made for the Silva. The younger Michaux had previously mentioned a cultivated "specimen in Mr. Pierce's arboretum, Chester county, Pennsylvania," and had noted a fact observed a number of times since, that the species blooms in both spring and fall.

In 1818 Nuttall states under *Magnolia cordata:* "Flowers yellow; appearing twice in the year in the garden of Mr. Landreth of Philadelphia. Leaves subcordate-oval, never truly cordate." There is a specimen from Nuttall's North American Herbarium in the New York Botanical Garden labelled *Magnolia cordata*, South Carolina (no other data). It is an excellent specimen, apparently typical for the species: twigs and leaves pubescent; flowers only 5 cm. long.

Elliott (1824) adds to the Michaux description: "Leaves of the calyx small." He evidently had seen the wild plant, for he continues: "Grows in the upper districts of Carolina and Georgia, more common around Augusta, than in any other part of the country with which I am acquainted."

Curtis (1860) evidently confuses the tree with *acuminata* var. *aurea*, as he gives it as occurring from the mountains of Ashe County, N. C., to Georgia, and his description is drawn entirely from Michaux.

Chapman (1897) gives the distribution as "Western parts of S. Carolina (*Michaux*), and Columbia County, Georgia, very rare." This is at present our only record from Columbia County. In all the editions of his Flora, Chapman includes both *cordata* and *acuminata*, but his distinctions do not hold well. In the last edition just cited he calls the flowers of both of them "yellow," which shows that he had in mind for *acuminata* the yellow-flowered southern mountain form now called var. *aurea*.

As far back as 1891, Sargent in his Silva, after discussing the cultivated form of *M. cordata*, says: "the exact counterpart of the cultivated plant is not known in a wild state. Forms approaching it in the shape and texture of the leaves, and in the size and color of the flowers, are occasionally found, however, on the Blue Ridge in Carolina and in central Alabama." As mentioned above, neither of the Michauxs made a distinction between the mountain and the sand hill forms in their treatment of *cordata*. Rehder (1940) also includes both *acuminata* and *cordata*, but he does not mention a southern yellow-flowered form of *acuminata*. For distribution of *cordata* he gives only Georgia.

Aside from the records of Nuttall, Elliott, and Chapman (and specimen from L. R. Gibbes from "South Carolina," in the New York Botanical Garden, noted below), none of which do I find mentioned by any writer since their time, *M. cordata* had remained, as Harper says, a mystery for at least 110 years. I may say here that while we cannot now consider the tree a mystery, we will still have to call it more or less of a puzzle, as will appear farther on.

The first new light that appeared in the knowledge of *M. cordata* came in 1913 when Mr. L. A. Berckmans of Augusta, Ga., found it at two places in Richmond County, Spear's Plantation and Goshen Plantation, a few miles south of that city. Dr. C. S. Sargent of the Arnold Arboretum (1914), in reporting this discovery, says: "The plants are growing in upland Oak and Pine woods, the largest of them being seven or eight feet tall. The plants begin to flower when not more

than three feet high, and in April of this year some of these shrubs bore forty or fifty flowers" (see also Sudworth, 1927). In a much earlier discussion of *M. cordata*, in which he had made an interesting comparison between the cultivated *cordata* and *M. acuminata*, Sargent (1886) first proposed the name *M. acuminata* var. *cordata*. This is the treatment he used in his Silva and the first edition of his tree Manual. The rediscovery of the feral tree caused him to change his mind and in the revised edition of his Manual he treats the tree as a species, but does not mention the yellow form of the mountains, which had previously so puzzled him. As far back as 1844 Loudon had considered *M. cordata* as cultivated in England as only a variety of *M. acuminata*.

In 1916 W. W. Ashe collected very typical specimens of the tree on the Spear place near Augusta, Ga., one of the stations where Berckmans had found it. These collections may now be seen in the herbaria of the University of North Carolina and New York Botanical Garden.

In 1917 Mr. J. S. Holmes, State Forester of North Carolina, found what we are now calling *cordata* in Anson Co., N. C., which adjoins the South Carolina line (Herb., University of N. C.).

In 1932 and 1933 Professor J. H. Miller of the University of Georgia discovered *cordata* in two places in Oconee County, Georgia. Since then Professor W. H. Duncan of the University of Georgia has collected it in Clarke, Putnam, and (?) Oglethorpe Counties in Georgia. We are much indebted to both of these gentlemen for furnishing us with excellent specimens. It was from one of Dr. Miller's plants that the drawing of *M. cordata* was made for our book (Coker & Totten, 1934, 1937).

In addition to these Georgia collections we have what we consider authentic records from other Georgia stations in Richmond, Columbia, McDuffie, Hancock and Bibb Counties.

Aside from the Michaux station on Horn Creek (now Edgefield County) the Elliott report, and the Nuttall and Gibbes specimens (no location other than South Carolina given), there is no record of a South Carolina discovery until Ashe in 1931 found *M. cordata* on the road to Graniteville across the river from Augusta.

In 1939 Mrs. J. D. F. Monts called attention to a small tree on the Monts farm in Richland County, South Carolina, about 14 miles north of Columbia. Notice of this appeared in the Columbia *State*, Aug. 9, 1939. Soon after this a number of trees were found in Newberry County, S. C., on the Nichols (or Lester brothers) land near Beaverdam Creek. All of these except one are small trees, some of them hardly more than seedlings. One, on the contrary, and unfortunately for the comfort of the taxonomist, is a very large tree, the largest at present known in the *Tulipastrum* group outside of the mountains. It is 20 inches in diameter at breast height and about 85 feet tall.

On a recent trip to Columbia (April 1942) the writer with Professor E. C. Coker of the University of South Carolina, Mr. C. H. Schaeffer, assistant state forester, and Mr. Fitzhugh McMaster visited this Newberry County station (April 18) and the writer brought back two small shoots which are now growing

on the grounds of the University of North Carolina. Professor E. C. Coker had previously brought in from this same location a young plant of the magnolia which at this time was blooming in the garden of the University of South Carolina although only 5½ feet high. (A News Release referring to this trip and other reports of *M. cordata* were sent out by Mr. Schaeffer on April 27, 1942, from the Columbia Office.)

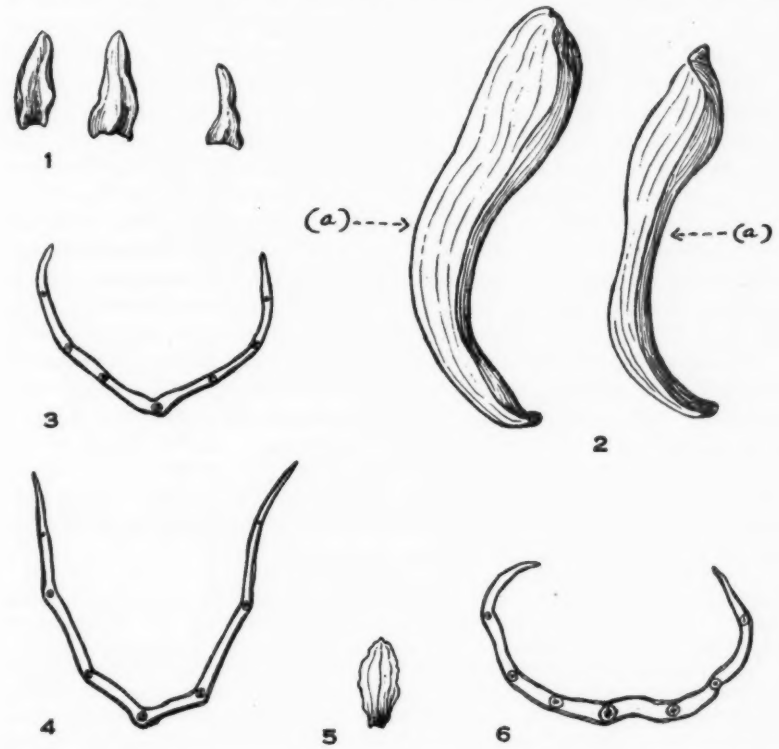
On the same afternoon Professor E. C. Coker, Mr. Schaeffer, and the writer went to the Monts farm to see the small tree above mentioned. It was in full bloom and buds just opening were carefully packed to be photographed. On our return to Chapel Hill two days later, the petals unfortunately had all opened and fallen off. This falling of the petals on almost the same day as the opening is characteristic of the *acuminata-cordata* group. Our photograph published herewith is from a specimen from this tree pressed at the time of the visit.

Prof. E. C. Coker has recently found several stations in the near vicinity of Columbia, from one of which we now have specimens. These are all represented on the map. Prof. Coker writes that at one of the stations, a hillside on the Du Bose place, there are 40 or 50 trees in an area of a few acres. Other South Carolina stations shown on the map have been reported to us by Mr. Schaeffer and Prof. Coker.

The extension of range into North Carolina in 1917 by Mr. J. S. Holmes has already been mentioned. In the following year Ashe says that *M. cordata* has been recorded from the central part of North Carolina in the extreme northwestern corner of Moore County or the adjoining part of Montgomery. We have not been able to confirm this record. Both of these stations are located on the extension of about the same topographical line and geographical area as Aiken and Columbia. It is the lower limit of the piedmont which is often referred to as the "fall line."

It may not be out of place to mention some other interesting plants that are rather closely associated with *M. cordata* along this fall line. In a letter of May 15, 1942, Mr. C. D. Beadle of Biltmore, N. C., writes that many years ago he and Mr. Cuthbert of Augusta found *Magnolia cordata* on the Falls of the Savannah River near Augusta—"near the magnolias was growing the rhododendron named by Small *R. Cuthbertii*, now our *R. minus* Mx." This rhododendron also occurs near Columbia and on May 5, 1937, Prof. E. C. Coker and the writer went to see a fine lot of it on the Le Conte farm about 10 miles east of the city. Here, near the rhododendron at the foot of a slope near a little stream we found a small tree of *Magnolia pyramidata*, a species never before reported from the state. At the top of the slope above this tree we also found the Black Sloe or Hog Plum (*Prunus injucunda*) known before only from one station in South Carolina, in Edgefield Co. about 20 miles from where Michaux found *M. cordata* (Boynton, 1902). The Le Conte farm is only about 6–7 miles from one of our stations for *M. cordata*. A short note on our trip to this farm was published in the Columbia *State* of May 25th, 1927. Another rare plant found growing in near proximity to *M. cordata* in Richland and Newberry Counties, S. C., and in the vicinity of Athens, Ga., is Carolina Buckthorn (*Rhamnus caroliniana*).

Magnolia acuminata var. *aurea* Ashe, as it occurs in the Blue Ridge Mountains of North Carolina, is a large tree with small flowers about 4.5–5 cm. high; sepals small, unequal, green with yellow tint, the outer petals same color but slightly more yellow inside and toward the tip, inner petals clear golden yellow (Wax Yellow, Ridg.) tinted with green only toward the base. Leaves varying from



FIGS.1–4. Magnolia acuminata var. aurea. Fig. 1, sepals, showing variation in size in the same flower; fig. 2, outer (left) and inner (right) petals: figs. 3 and 4, cross sections cut at (a) of inner and outer petals, respectively, × 4.

FIGS. 5 AND 6. Magnolia acuminata var. cordata. Fig. 5, sepal; fig. 6, cross section of inner petal, × 4.

All figures except those noted are natural size.

broadly ovate with rounded to truncate base and short apiculus to elliptic with cuneate base and longer point, at maturity usually softly pubescent beneath and glabrous above except on the larger veins. Twigs glabrous, buds densely pale-pubescent; cones usually small and deformed, often maturing only a few follicles, sometimes only 1–3; flowering from late April to late May, depending on the altitude. From the typical form of *acuminata*, these trees differ only in the much

smaller, more yellow flowers, and perhaps in the smaller and more deformed cones. (See our photograph and drawings of flower of var. *aurea* from Macon Co., N. C. For a good photograph of the typical *acuminata* enlarged, see Rogers, 1935; copied in Collingwood, 1941.)

Magnolia cordata, as we include it on our map, differs from var. *aurea* so far as we have yet found, only in the usually smaller size of trees,[1] pubescent twigs of first and usually second years, somewhat more consistently smaller and more broadly ovate leaves and in geographical range (lower altitudes). The base of the blade is by no means specific; it may be broadly rounded or more or less often strongly pointed. The habit of blooming again in the fall may also be important. We have no data regarding this point in var. *aurea*. The name *cordata* is a misnomer, as the blades are only rarely even faintly cordate at base, as is also true in the cultivated form. In fact they have no more tendency to this than the var. *aurea* or even the typical northern form. The writer has recently gone over the magnolias of this group in the New York Botanical Garden and finds that the leaves of typical *acuminata* are quite often broadly ovate with abruptly rounded or truncate base, as for example specimens from New York, West Virginia, etc. The only one of the *Tulipastrum* group in which the leaves may be really cordate is *M. acuminata* var. *ozarkensis* of which we have ample specimens. For this and other varieties see Ashe (1931). In this article Ashe describes a new variety, *alabamensis*, of *acuminata* to include the Alabama plants he had previously (1927) referred to *cordata*. Mohr's report of var. *cordata* may be this variety *alabamensis* or possibly var. *aurea*.

We think it worth while to quote here a few lines from Loudon regarding the typical *M. acuminata* as it occurred in cultivation in England in 1844 (note the leaf variations and early flowering from layers):

> The leaves are from 6 in. to 7 in. long, and from 3 in. to 4 in. broad, upon old trees, but double that size upon young vigorous-growing plants. Michaux describes them as oval, entire, and very acuminate; but, in the seedlings raised in British nurseries, they are found sometimes ovate, nearly orbiculate, and cordate-acuminate. The flowers, which are 5 in. or 6 in. in diameter, are bluish, and sometimes white, with a tint of yellow. They have but a feeble odour; though, as they are large and numerous, they have a fine effect in the midst of the superb foliage. Plants raised from seeds do not usually produce flowers till they are eight or ten years old, when the tree will probably be from 15 ft. to 20 ft. in height; but plants raised from layers produce flowers in two or three years.

Loudon's two plates illustrating *acuminata* (pl. 7) and *cordata* (pl. 9) show very well the differences between the typical large-flowered *acuminata* with larger, more elliptical leaves, and the small-flowered *cordata* with broader and more rounded leaves.

In summing up all the evidence we have as to the position of our lower piedmont tree in the *Tulipastrum* group of magnolias, we arrive at the disappointing

[1]The big tree in lower Newberry Co., S. C., is about 85 ft. high and 5 ft. 7 in. in circumference (Schaeffer); the record from upper Newberry Co. is of shoots from a stump 12 inches in diameter and the Clark Co., Ga., tree is 50 feet tall and 9 inches in diameter.

conclusion that *cordata* is only a rather vaguely defined, marginal extension of the southern yellow-flowered variety of *M. acuminata*. It is now evident that we cannot find a clear-cut geographical distribution for *cordata* as distinct from var. *aurea*. Its extension to the Athens, Ga., area brings it much closer to the mountains. The specimens from Clarke and Oconee Counties, Georgia, are good *cordata*, but the one from Oglethorpe County, adjoining, has glabrous twigs and a large cone (6.3 cm. long when dry), though the leaves are all typical *cordata*, very broad, one even suborbicular, with blunt bases and very short abrupt points. The tree is about 18 inches in diameter and 35 feet tall. We are indicating this station with a ? on our map. We now have a collection from Spartanburg Co., S. C., in the upper piedmont that might just as well be called *cordata* as var. *aurea*. It has small yellow flowers and *pubescent* twigs and rather narrow cuneate leaves. The farthest from the mountains that we know of for good var. *aurea* is not far from Greenville, S. C., on the Caesar's Head road, found by the writer growing so close to a young tree of *M. Fraseri* that the branches were touching. It was a small tree with yellow flowers and *glabrous* twigs. We are evidently dealing with a very variable group with extremes connected by a series of forms. This being so we can hardly be justified in calling the lower piedmont (fall line) tree anything more distinct than a variety, as Loudon long ago decided for the cultivated tree and as Sargent treated it in his first publications. This variety may be described as follows:

Magnolia acuminata var. **cordata** Sarg.

A small tree rarely over 25–30 feet high and 8–10 in. in diameter, usually much smaller, rather symmetrically oval when not crowded; bark of trunk brown and deeply and closely ridged as in *M. acuminata;* terminal foliage buds in winter condition narrow, up to about 1.2 cm. long, and 3.5 mm. wide; lateral buds shorter and plumper; flower buds ovate, bluntly pointed, about 6–7 x 10–13 cm.; all buds densely white-tomentose; leaves typically ovate-elliptic, with or without a short abrupt apiculus, the base broadly rounded, usually about 8–13 cm. long, and 6–8 cm. broad (up to 15 cm. long and 10 cm. broad); under-surface closely pubescent, upper surface smooth at maturity, both surfaces densely pubescent when young, rather thick at maturity; petioles typically short, usually 1–1.5 cm., on strong shoots up to 2.5 (3) cm., minutely pubescent; twigs of the season pubescent throughout the year, also usually pubescent or at least roughened with the bases of hairs, on twigs of the preceding year; blooming in mid-April and again in the fall; flowers usually about 5 cm. high, and 5 cm. broad, petals 6, nearly upright from a curving base; outer petals broader, strongly cupped and folded inward, inner petals about the same shape, but less cupped; color of inner petals a clear golden yellow (Wax Yellow of Ridgway); color of outer petals less deep than inner (about citron yellow); calyx lobes glabrous, reflexed, more or less unequal, up to about 9 mm. long, 5 mm. broad, about color of outer petals; cone nearly always small and knotty, due to few follicles maturing, usually 2.5–3.5 cm. long; seeds orange-scarlet, about 1 cm. long, 8 mm. broad, maturing sometime in late August (some cones are so small that they have only one or two follicles maturing); inner bud scales brown, very thin, translucent, about 2 cm. long, glabrescent, thinly appressed-hairy toward the tips.

Confined to lower piedmont section from lower central N. C. to central S. C., central and upper middle Ga.; scarce and local, usually found on wooded hillsides along smaller streams together with the usual deciduous trees of the section. Pines, also, may not be far away.

DEPARTMENT OF BOTANY,
UNIVERSITY OF NORTH CAROLINA,
CHAPEL HILL, N. C.

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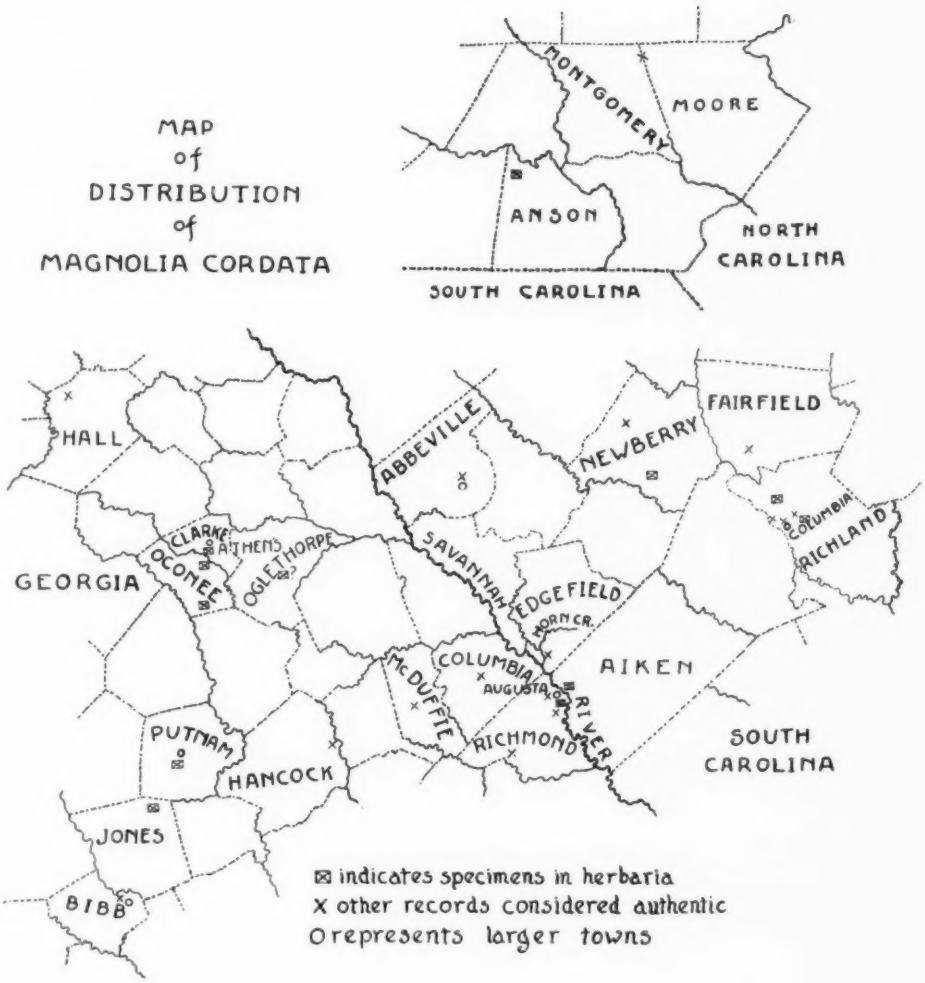
MAP
of
DISTRIBUTION
of
MAGNOLIA CORDATA
MONTGOMERY
MOORE
ANSON
NORTH
CAROLINA
SOUTH CAROLINA
HALL
ABBEVILLE
NEWBERRY
FAIRFIELD
COLUMBIA
RICHLAND
CLARKE
ATHENS
OCONEE
OGLETHORPE
GEORGIA
SAVANNAH
EDGEFIELD
HORN CR.
McDUFFIE
COLUMBIA
AUGUSTA
AIKEN
RIVER
PUTNAM
HANCOCK
RICHMOND
SOUTH
CAROLINA
JONES
BIBB
⊠ indicates specimens in herbaria
X other records considered authentic
O represents larger towns

PLATE 18

MAGNOLIA ACUMINATA VAR. CORDATA

Richland County, S. C., April 18, 1942. Photograph of herbarium sheet showing flower, × ⅔

PLATE 19

MAGNOLIA ACUMINATA VAR. CORDATA

Richland County, S. C., Aug. 8, 1939. Photograph of herbarium sheet showing two small open cones and seeds.

PLATE 20

MAGNOLIA ACUMINATA VAR. AUREA

Macon County, N. C., May 25, 1942. One petal removed from lower flower. × ½

A NEW RAFINESQUE LETTER

By Phillips Russell

A hitherto undiscovered and unpublished letter, amounting almost to an autobiography, of Charles S. Rafinesque has been found in an old letter file belonging to the archives of the University of North Carolina at Chapel Hill. It consists of an application for the then vacant professorship of modern languages in that institution. It was written in 1826, the same year[1] that this eccentric scientist of the early 19th century in America departed angrily from Lexington, Ky., where he had been a member of the faculty of Transylvania University, and returned to Philadelphia, where he eventually died in the bitterest poverty.

If no other evidence existed, this letter would be sufficient testimony concerning certain Rafinesque traits: his tendency to be temperamentally difficult, his inclination to be boastful, and his pride in his own versatility. It is also proof that Rafinesque had not the complete knowledge of the English language of which he boasts, for the style is that of a foreigner.

The letter, which was addressed to Ichabod Wetmore of Raleigh, N. C., chairman of a trustee committee appointed to choose the new professor, is herewith printed so as to show Rafinesque's own spelling, grammar, punctuation, underscoring, and capitalization. It will be noted that he was misled by the address of the chairman into thinking that the University was located at Raleigh.

Lexington, Ky 22d April 1826

I.Wetmore Esqe
Raleigh N.C.

Sir—I write you in consequence of having seen an advertisement in the National Gazette, concerning a Vacancy of the Professorship of Modern Languages in the University of N. Carolina.

I beg leave to apply for that appointment. The claims which I can state in my behalf are the following. I have been for six years Profr of Modern Languages in the Transylvania Univy of Lexington. I have taught there the french, Spanish & Italian, but I am going to leave this Institution where I have not been adequately rewarded.—I was born of a french father & a german Mother. I was raised in France and Italy; I have been in Spain & a portuguese Colonys—I can teach the French, Spanish, Italian & Portuguese. I have not merely a *familiar acquaintance* with those languages; but a *complete mastership* of the French, Italian, and English, so as to be able to write in them fluently both in *prose* and in *verse*. My knowledge of Spanish & Portuguese is more limited, for want of practice; but I know those languages and their grammar as well as our Teachers of greek & Latin know these. I have also a general knowledge of Latin, greek &c, and a smattering of hebrew, Arabic, German &c. To which I may add a philological knowledge of comparative Linguistics or all languages compared

[1]It is erroneously stated by R. E. Call, *Life and Writings of Rafinesque*, pp. 49 and 119, that Rafinesque left Kentucky in 1825, although the year is correctly given on p. 42.

It may be of interest to readers that while Rafinesque did not obtain the position applied for it was given to another naturalist, Nicholas M. Hentz, the famous student of spiders. —Ed.

together—as can be proved by my printed Table of the compared Roots of all Languages.

I have written several works & Pamphlets, upwards of twenty, in french, Italian, English & Latin—On Arts & Science, Literature, &c—and I have by me many additional manuscripts, among which a Comparative Grammar & Vocabulary of the French, Italian, Spanish & Portuguese, besides several of those Languages & Dialects. I know their Literature &c I can read, construe, translate &c from each into each other.—The English I know completely also, having come young into America, & I speak it without a foreign accent. The french I speak purely, without local accent. The Italian I speak with the purest Tuscan & Roman Accent &c I can deliver Lectures extempore in French, Italian & English. I can even compose extempore verses or *Improvisare* in Italian. And I can sing in Italian, french, Spanish, English, &c.

These qualifications embolden me to offer my services to the University of North-Carolina, as a Professor of Modern Languages, which I can fill with skill and credit.—Altho' your Advertisement does not state that references are needed, yet as this is often a requisite, I can in that case give satisfactory references & evidences of my attainments.

I have one hundred friends who could recommend me, and if high names may have any influence, I can refer to Dewitt Clinton of Newyork, Dr Samuel L. Mitchell of Do—Zaccheus Collins & William Rawle of Philadelphia—Dr James Smith of Baltimore—Henry Clay & Timothy Winn, Washington City, who all know my attainments & standing.

I am also known to them & the public as a Botanist, a Naturalist, a historian, an antiquarian, an Engineer & a Draftsman—I have never visited North Carolina & should wish to explore it also as a Botanist, historian &c: this is an additional Inducement for my application.

I began this letter in Lexington & perhaps shall conclude it at the harrodsburg Springs where I am going on a short visit. I propose removing to Philadelphia next month, with my Library & Cabinet which has already been sent on packed up in 40 boxes & Trunks.—Your answer must be directed to *Philadelphia*. where I shall be in June, after an exploring Journey throuth Ohio & Virginia.

Hoping that what I have stated may prove satisfactory, I conclude by advising you that my Statements have been fair and true. If any doubts are felt, I am willing to be appointed for only one year, so that we may all know whether we suit as well as contemplated, & and that I may know whether Raleigh will prove sufficiently agreeable for a permanent Residence, as I hope it may; but I thought so of Lexington once, and great promises were held to me, which have been broken; and I have had to move & remove with my large apparatus & Library at great Expence; which I wish to avoid in future.

I remain very respectfully yours—

C. S. Rafinesque
A.M.—Ph.D. &c

(Postmarked Harrg. K(y) Apr 27)

BOOKS FROM CHAPEL HILL

TREES OF THE SOUTHEASTERN STATES. By W. C. COKER and H. R. TOTTEN. Second Edition. All the trees which grow naturally in Virginia, North Carolina, South Carolina, Georgia, Tennessee, and northern Florida are included. The more important naturalized trees are also included, as well as a list of native shrubs which occasionally reach tree size. Intended for the use of anyone interested in trees, whether high school student, college student, botanist, or amateur. About 417 pages and 248 illustrations. 4¼″ x 7¼″. $2.00.

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THE BOLETACEAE OF NORTH CAROLINA. By WILLIAM CHAMBERS COKER AND ALMA HOLLAND BEERS. This study is based on notes and photographs taken by the senior author over a period of about forty years. For a good many years both authors have worked on the group and have made many collections. While most of the work has been done in Orange County and in the Blue Ridge and Alleghany Mountains of North Carolina, many specimens have been collected in South Carolina and Georgia. While the title covers only North Carolina, this work includes all except the few species reported from the neighboring states of Virginia, South Carolina, Georgia, and Alabama of which examples have not been available or that have been reduced to synonomy with species included.

There are described under the genus *Boletus* sixty-eight species and six varieties. Of these, three species and four varieties are described as new. Four species of *Boletinus* and one of *Strobilomyces* are also included. Twenty species are illustrated in color and nearly all of the others by halftone photographs.

There are 96 pages of text; 6 colored plates; 55 halftone plates; and 5 plates of line drawings showing microscopic details. $7.00.

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